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CURRENT CANCER RESEARCH

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CELL DIFFERENTIATION, TUMOR GROWTH, INVASION, AND METASTASIS

OCTOBER 1981

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CURRENT CANCER RESEARCH

I. REGULATION OF TUMOR CELL DIFFERENTIATION AND GROWTH

A. GENETIC AND EPIGENETIC MECHANISMS IN DIFFERENTIATION AND GROWTH

1. GENETIC REGULATION OF DIFFERENTIATION AND PROLIFERATION

1. THE MOLECULAR BIOLOGY OF INITIATION OF DNA BIOSYNTHESIS

Shall, S., University of Sussex, Biochemistry Lab., *Sussex, Brighton BN1 9QG, England, United Kingdom*

OBJECTIVE: The objective is a molecular understanding of the frequency of cell division. The rate-determining step is at or just before the initiation of DNA replication. We propose to discover the rate-determining process by a molecular analysis of the initiation of DNA replication, which will include a study of the molecular factors controlling initiation. Thus, our immediate objectives are to isolate and study those DNA sequences which are origins of DNA replication. We propose to sequence the isolated origins and then to establish an in vitro, cell-free assay for the initiation of DNA replication.

APPROACH: The approach is two-fold. In the first instance we shall isolate the DNA made in the first 1% of the S phase by density-labelling, and then separation by isopycnic centrifugation. We will then purify the 'origin' DNA and clone it in a suitable vector. After amplification we will determine the sequence of this DNA to establish if there is a common element of 'origin' DNA. Our second approach is to use yeast as a host for a specially constructed plasmid that will be an assay for putative origins of replication. In this manner we intend to isolate origins of replication from several different species. These will be amplified in E. coli and sequenced. Then we will determine whether there are features common to these origins. We will also determine whether the putative origin fragments have specific structural features. The last part of this project will be to develop a cell-free DNA-initiation assay.

2. CONTROL OF CELL REPLICATION

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OBJECTIVE: To identify mRNA molecules and subsequently genes that produce proteins involved in the regulation of DNA replication and the passage of the mammalian cell through the cell cycle.

APPROACH: We are cloning cDNA copies of polysome-associated, poly A-containing mRNA molecules in order to construct a library of expressed mammalian genes. This library will be screened in various different ways to identify clones corresponding to potentially interesting sequences.

PROGRESS: A few clones have been obtained so far.

3. A CORRELATED STUDY ON THE BIOLOGY OF NEOPLASIA

Baserga, R. L., Henderson, E. E., Ming, P. L., Duker, N. J., Ordahl, C. P., Tilghman, S. M., Temple University, School of Medicine, Dept. of Pathology, *3322 Broad St., Philadelphia, Pennsylvania*, 19122, United States of America

This is a continuation application for a Program Project that brings together a number of investigators interested in the basic biology of neoplasia. The two basic cellular processes that determine the growth of a tumor are cell proliferation and cell death. Accordingly, this Program Project brings together investigators interested in the basic mechanisms of cell proliferation and cell death in both normal and neoplastic cells.

The program is based on the interaction of individual scientists with each other and with a variety of approaches including gene cloning, the introduction of macromolecules into viable cells, transfection in order to alter the genotype, as well as a variety of other more established projects. The goal is the identification of genes that play a role in cell division and cell death.

4. GENE CONTROL OF MAMMALIAN DEVELOPMENT AND DISEASE

Mintz, B., Fleischman, R. A., Wagner, E. F., Stewart, T. A., Karin, M., Inst. for Cancer Research, *7701 Burholme Ave., Philadelphia, Pennsylvania*, 19111, United States of America

The broad objectives of the program are to identify genetic mechanisms underlying normal mammalian differentiation; to define the developmental changes resulting in specific genetic diseases, and to create mouse models of human diseases; and to analyze the developmental basis for certain malignancies. Genetic markers will be used as probes. They will be introduced into mouse cells with developmental potential, in three experimental approaches: In the first, predetermined nuclear or mitochondrial mutational changes will be incorporated into euploid, developmentally totipotent, mouse teratocarcinoma stem cells in culture, by selecting or screening mutagenized cell populations for the mutation of interest; further development of the cells will be obtained by introducing them into early embryos. Among the nuclear-gene mutants planned for study will be those coding for specific cell-surface receptors, in order to analyze receptor-mediated pathways and their ontogeny as an important feature in specialization of cell function. In a second experimental approach, exogenous recombinant cloned genes, in either native or modified form, will be introduced into mouse embryos or into cultured cells with developmental potential (e.g., teratocarcinoma cells), for the purpose of analyzing tissue-specific control of gene expression during differentiation, and to correct genetic defects. The initial emphasis in these experiments will be on globin genes and hemoglobinopathies in erythroid development. In a third approach, also centered around hematopoietic development, genetically marked pluripotent hematopoietic stem cells of embryonic or fetal origin will be introduced into early fetuses at the outset of hematopoiesis; donor-derived cells are thus processed through allogeneic host organs throughout development. These experiments are aimed at analyzing developmental lineages, cell interactions and acquisition of specificities in erythroid and lymphoid cell maturation, control of globin gene expression, and the developmental basis of defects in various mouse anemias, autoimmune disease, and strain-specific malignancies.

BIBLIOGRAPHIC REFERENCES: Mintz, B. (1979). Teratocarcinoma cells as probes of mammalian differentiation. In: Mechanisms of Cell Change (J. Ebert and T. Okada, eds.), 119-127. John Wiley and Sons, Inc., New York. Mintz, B. (1979). Teratocarcinoma cells as vehicles for introducing mutant genes into mice. Differentiation 13, 25-27.

5. REGULATORY MECHANISMS IN TUMOR BIOLOGY

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This program-project inquires into the basic mechanisms which regulate the replication and differentiation of normal and cancer cells. A combination of genetical, biochemical and cell biological approaches is used to address the following problems:

1. An elucidation of the mechanisms by which RNA polymerases bind to defined DNA templates and carry out a regulated transcription of specific genes. 2. The clarification of the role of gene structure and other gene products in the regulation of transcription. 3. A description of the molecular reactions by which primary gene transcripts are processed into functional messenger RNA species. 4. The identification of the components in nuclear envelopes and the definition of their roles in the regulation of nuclear function. 5. The identification of the active components of the DNA replicase system and a definition of the processes by which DNA-chromatin complexes are replicated. 6. The development of genetic systems in eucaryotes (i.e., Physarum polycephalum and mouse teratoma cells) for the study of the regulation of cell replication and differentiation.

BIBLIOGRAPHIC REFERENCES: Mueller, G. C., Kajiwara, K., Kim, U. H., and Graham, J. Proposed Coupling of Chromatin Replication, Hormone Action, and Cell Differentiation. Cancer Res., 38: 4041-4045, 1978. Mueller, G. C. and Kim, U. H. Displacement of Estradiol from Estrogen Receptors by Simple Alkyl Phenols. Endocrinology, 102: 1429-1435, 1978.

6. GENETIC ANALYSIS OF HUMAN MALIGNANCY

Stanbridge, E. J., University of California, Irvine Campus, California College of Medicine, Dept. of Microbiology, *Irvine, California*, 92664, United States of America

OBJECTIVES: To extend our studies of the genetic control of the transformed and tumorigenic phenotypes in human cells. This includes analysis of chromosomal and extrachromosomal control of the expression of these phenotypes and determination of markers specifically associated with the tumorigenic phenotype.

BIBLIOGRAPHIC REFERENCES: Doersen, C. J. and E. J. Stanbridge. 1979. Cytoplasmic inheritance of erythromycin resistance in human cells. Proc. Natl. Acad. Sci. USA 76:4549-4553.

7. CONTROL OF CELL DIVISION IN THE NEMATODE C ELEGANS

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The development of the small soil nematode Caenorhabditis elegans involves a rigidly determined and precisely known pattern of cell divisions. We hope to elucidate the cellular and genetic mechanisms that control this pattern of cell divisions as well as subsequent cell fate in C. elegans. We will attempt to establish the relative roles of cell autonomous and cell interactive factors, and the ways in which these factors act. We also hope to reveal aspects of the underlying genetic program that specifies cell lineage and cell fate. Our proposed studies combine physical and genetical ablation techniques to explore cell-cell interactions (how does elimination of one cell influence the fate of another?) and detailed genetical, and in some instances biochemical, studies to determine the roles of particular genes in development. One major approach will be the isolation and characterization of mutants should help establish the relationship between lineage history and cell fate, as well as reveal the specificity with which given genes influence particular cell divisions. To explore the complexity of the genetic program controlling cell division patterns, we will identify all genes that affect a specific set of cell divisions, those involved in the development of the vulva. For a limited number of genes, we hope to determine, ultimately at the molecular level, both the normal function of the gene and the ways in which mutations alter or disrupt that function. In addition, we will examine the possibility that programmed cell death provides a mechanism for controlling cell division. We will also seek interspecific cell lineage differences (as we have already found between C. elegans and another nematode, Panagrellus redivivus) and attempt to determine the genetic bases of these differences.

BIBLIOGRAPHIC REFERENCES: Hodgkin, J., Horvitz, H.R. and Brenner. Nondisjunction mutants of the nematode Caenorhabditis elegans. Genetics 91: 67-94 (1979). Horvitz, H.R., Brenner, S., Hodgkin, J. and Herman, R. A uniform genetic nomenclature for the nematode Caenorhabditis elegans. Molec. gen. Genet. 175: 129-133 (1979).

8. ANALYSIS OF CELLULAR REGULATORY MECHANISMS

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This research project is directed towards an understanding of the genetic logic and biochemical mechanisms which regulate cell proliferation and differentiation. This goal will be approached through the genetic and biochemical analysis of developmental mutants - lesions which cause either abnormal cell death, morphogenetic deformities, misapplication of determinative potential, uncontrolled neoplastic cell growth, or early developmental lethality. Because of the large genetic repertoire of mutants and methods Drosophila melanogaster will be used as the experimental organism. The research will be directed toward the isolation and characterization of new morphogenetic mutants in Drosophila melanogaster and the development of a system to utilize such mutants, as well as existing mutants, in a biochemical study of the control of gene expression.

9. MECHANISMS OF SEX DIFFERENTIATION

Haseltine, F. P., Gordon, J., Yale University, School of Medicine, Dept. of Obstetrics & Gynecology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

The goal of this investigation is to examine the genetic events underlying sex differentiation. One group of studies will focus on the production and characterization of mosaic mice in which the sex chromosome balance is experimentally altered. The two initial objectives of these studies are to produce mosaic mice whose sex karyotypes are predetermined, and to develop methods for assaying the tissue distribution of karotypically male and female cells in neonatal mosaic mice. On this basis, it should be possible to analyze systematically the interactions between germ cells and their embryonic environment in the course of gonad development, and in particular to study in detail the perturbation of this development that occurs in sexually mosaic or aneuploid fetuses.

The other aspect of this investigation concerns the effects of these factors on the alternation of normal gonad development that leads to the formation of teratomas. Finally, these interactions will be examined directly by following the development of teratoma-derived cells introduced into normal blastocysts.

10. GENETIC ELEMENTS CONTROLLING CELLULAR MULTIPLICATION

Pitot, H. C., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

This is part of a broader project. A summary of this subproject is not available.

11. POSSIBLE ROLE OF THE EPIGENETIC CONTROLS INVOLVED IN NORMAL CELL DIFFERENTIATION IN MAINTAINING A NON-NEOPLASTIC PHENOTYPE

Cooper, P. D., Marshall, S. A., Australian National University, John Curtin School of Medical Research, Dept. of Microbiology, *P.O. Box 334, Canberra, A.C.T. 2601, Australia*

OBJECTIVE: To investigate mechanisms of carcinogenesis in terms of perturbation of normal cell differentiation.

APPROACH: Epigenetic processes known to be active in normal cell differentiation (extracellular matrix, cell surface effector/receptors, biopotentials, microfilaments, etc.) are deliberately perturbed in vitro and in vivo to look for cell 'transformations' of various kinds.

PROGRESS: Certain combinations of treatments have produced foci of 'transformed' cells in mouse cell cultures. The characters of these are being explored, and the question asked of whether the foci arise for the reasons postulated in 'approach'.

12. NORMAL DIFFERENTIATION AND CARCINOGENESIS

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Several lines of evidence supporting the theory of nuclear equivalence are consistent with the view that specialized cell types of adults contain the complete set of genes derived from fertilized eggs; however, conclusive proof is still lacking. Am-

phibian nuclear transplantation has been the most stringent test of this theory. When nuclei from early embryos are transplanted into eggs, the eggs develop normally. However, only a few nuclei from normal adult cells or from the renal carcinoma (herpesvirus etiology) promote eggs to develop into abnormal tadpoles. In the previous grant, I proposed that nuclei from advanced cell types might be conditioned in oocytes and their genetic potential enhanced. We have established that this hypothesis is tenable. First, blastula nuclei injected into oocytes support development through embryogenesis, indicating that the oocyte can be used to test the developmental potential of nuclei. Second, nuclei from non-cycling and terminally differentiated erythrocytes that do not synthesize DNA in eggs will reverse and synthesize DNA following conditioning in oocytes, indicating that the critical test of the theory of nuclear equivalence can now be pursued in the oocyte. The current application proposes: 1) to test the developmental potential of nuclei from determined and specialized cells and from the renal carcinoma by first conditioning nuclei in oocytes; and 2) to investigate mechanisms responsible for the reversibility of nuclei conditioned in oocytes. If a significant number of normal frogs develop, the nuclear totipotency of normal specialized cells and the renal carcinoma would be demonstrated. Such results would: 1) prove the theory of nuclear equivalence in normal differentiation; and 2) demonstrate that viral carcinogenesis of this tumor has an epigenetic origin and the cells can therefore be reversed from malignancy to normalcy with proper environmental conditions.

BIBLIOGRAPHIC REFERENCES: DiBerardino, M.A. Nuclear and chromosomal behavior in amphibian nuclear transplants. Intern. Rev. Cytol. Suppl. 9, pp. 129-160 (eds. DiBerardino and Danielli), in press, 1979. Academic Press New York and London. DiBerardino, M.A. and N.J. Hoffner. The current status of cloning and nuclear reprogramming in amphibian eggs. In Differentiation and Neoplasia, Third International Conference on Differentiation (eds. McKinnell, DiBerardino and Blumenfeld). in press, 1979. Springer-Verlag, New York and Berlin.

13. KELOIDS - AN IN VITRO MODEL OF TUMOR GROWTH REGULATION

Russell, J. D., Russell, S. B., Trupin, K. M., Meharry Medical College, School of Graduate Studies & Research, Dept. of Microbiology, *1005 18th Ave. N., Nashville, Tennessee,* 37208, United States of America

Keloids are dermal tumors which occur in response to trauma. A hereditary predisposition to Keloid formation occurs with high frequency in black populations. In addition to overproliferation of fibroblasts this tumor growth involves abnormal collagen metabolism.

The goals of these studies are to identify the primary gene defect causing this condition, its mode of action, and those environmental factors which alter its expression. The strategy used in this investigation is to search for patterns of differences in cell culture between fibroblasts derived from keloids and from normal scars and skin. We have found that cultured fibroblasts isolated from normal and keloid tissue are similar in regard to both growth characteristics and rate of collagen production; however, the addition of physiological levels of hydrocortisone to the culture media results in significant differences between these cell types in both their growth characteristics and rate of collagen production, suggesting an abnormality in the regulation of both collagen metabolism and growth in keloid cells.

The studies described here are aimed at elucidating the regulatory mechanism functioning abnormally in keloids and the relationship between the abnormal phenotypes observed, namely collagen metabolism and cell growth, by determining (1) the genetic and epigenetic contributions to these phenotypes; (2) whether the phenotypes are cell limited and the dominance relationships between them; (3) the mechanisms of the response to hydrocortisone; (4) the ability of other environmental variables to elicit or prevent differences between normal and keloid fibroblasts.

Results to date suggest coordinate regulation of several steps in collagen metabolism by hydrocortisone and that the abnormality in keloid cells affects a regulatory process common to several of these steps.

14. TERATOCARCINOMA AND ONCO-EMBRYONIC PHENOTYPE

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The overall objective of this proposal is to study the epigenetic factors that favor, modify or inhibit the expression of the malignant phenotype of undifferentiated embryonic cells and stem cells of teratocarcinoma. In a continuation of our present studies we plan to investigate the differentiation and growth of normal embryonic cells and embryonal carcinoma cells transplanted into adult hosts or injected into developing embryos as well as under controlled conditions in vitro. Differentiation and growth of embryonic cells and embryonal carcinoma cells will be primarily investigated as a function of interaction between the undifferentiated cells and somatic cells in the adult or embryonic cells in utero and in vitro. Chemical and biological approaches to modify the differentiation will be pursued. The genome of the embryonal cells will be manipulated to determine the effects of genomic alterations on expression of malignant phenotype. Differentiation and/or expression of onco-embryonic phenotype will be monitored by light and electron microscopy using enzyme histochemical and immunocytochemical techniques. These studies are intended to increase our understanding of basic mechanisms accounting for the transition of normal embryonic cells into stem cells of teratocarcinoma and the epigenetic control of malignancy in general.

15. CELL DIFFERENTIATION AND CANCER

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The present proposal is the result of the interaction of geneticists, embryologists, molecular biologists, cell biologists, immunologists and virologists of the Wistar Institute in studies of the relationship between cell differentiation and cancer and of the regulation of gene expression in stem versus differentiated cells.

A variety of experimental approaches and cellular systems will be used by the different investigators to probe erythroid, myeloid and lymphoid differentiation and to understand their relation to cancers of the hematopoietic system.

Mouse embryonic and teratocarcinoma cells will be used to determine what factors affect the transition from the normal embryonic to embryonal carcinoma (EC) cells and to determine whether the malignant behavior of EC cells is the result of epigenetic changes.

The mechanisms of control of gene expression in pluripotent and totipotent mouse and human teratocarcinoma-derived stem cells will be investigated with DNA and RNA tumor viruses as probes.

The use of monoclonal antibodies produced by hybridomas to study surface antigens expressed at different stages of cell differentiation will be stressed.

16. MUTATIONS AFFECTING GENE EXPRESSION IN TUMOR CELLS

Ivarie, R. D., University of Georgia, Franklin College of Arts & Sciences, Dept. of Molecular & Population Genetics, *Athens, Georgia,* 30601, United States of America

The objective of the proposed studies is to use somatic cell genetics to understand the mechanism of the regulation of cell growth and the control of gene expression in mammalian cells. We have chosen cultured rat pituitary tumor cells as a model system for this approach because the growth of these cells is regulated by a number of signals. In addition, the expression of a specific gene product, growth hormone, is regulated by many of the same signals. The latter property of these cells is particularly useful because, in parallel with effects on growth, highly specific biochemical probes measuring specific messenger RNA levels and specific gene copy number are available to facilitate analysis of the nature of mutant defects.

To isolate hormonally unresponsive cell lines, two properties of these cells will be exploited. The first is the stimulation of cell growth by L-triiodothyronine (T3). If enhancement of growth requires the induction of a specific gene product, unresponsive cells could have mutations throughout the t3-mediated pathway. The second property upon which mutant isolation

relies is the synthesis and secretion of growth hormone. Procedures will be developed to screen for the production of this polypeptide from 10 to the 5th power - 10 to the 7th power clones. Because the screening method is quantitative for growth hormone, mutations may be obtained at any of the steps leading to the induction, processing and secretion of the polypeptide. For a complete genetic analysis, the important feature of both selection methods is an eventual complementation analysis by somatic cell hybridization to determine the number of gene products, and the regulatory function, involved in t3-mediated regulation of growth and gene expression.

The biochemical defect suffered in each mutant will be thoroughly characterized with respect to: any protein detected on two-dimensional gel analysis; T3-receptor function; and the synthesis and secretion of growth hormone or its mRNA. The availability of probes for messenger RNA levels and gene copy number will provide additional help for analyzing the nature of mutant deficiencies.

17. TUMOR INCEPTION AND PROGRESSION

Meins, F., University of Illinois, Urbana Champaign Campus, School of Life Sciences, Dept. of Botany/Genetics & Development, *Urbana, Illinois*, 61801, United States of America

Directed, heritable alterations in gene expression, called epigenetic changes, have been proposed as a basic mechanism for tumor transformation. The major objectives of this project are: to find out to what extent epigenetic changes in the production of specific growth factors can account for autonomous growth, tumor progression, and tumor reversal in plants. The specific aims for the current grant period are: 1) to study the molecular basis for heritable changes in the cell division factor autotrophy of cultured tobacco cells: 2) to identify relationships between the nature of the association of Ti plasmid DNA sequences with the tumor cell and expression of autonomous growth; and 3) to use Vinca lines in culture to test the hypothesis that tumor progression involves a perturbation of cells followed by selection for more autonomous cell types.

BIBLIOGRAPHIC REFERENCES: Binns, A. N. and Meins, F., Jr. (1979), Cold-sensitive expression of cytokinin habituation by tobacco pith cells in culture. Planta 145., 365-369. Meins, F., Jr., Lutz, J., and Binns, A. N. (1980), Variation in the competence of tobacco pith cells for cytokinin-habituation in culture. Differentiation 16, 71-75.

18. CONTROL OF CELL DIVISION IN NORMAL VS MALIGNANT CELLS

Bello, L. J., Magne, J. B., University of Pennsylvania, School of Veterinary Medicine, Dept. of Pathobiology, *39th & Pine, Philadelphia, Pennsylvania*, 19104, United States of America

Malignant cells do not respond to the regulatory signals which control the proliferation of normal cells. Unfortunately, it is and will remain difficult to define the nature of the defect in malignant cells while so little is known concerning the control of cell division in normal cells. The object of this research is to expand our knowledge of cell division in both normal and malignant cells.

In this proposal we plan to determine the role of differential gene function in human diploid cells as they undergo the transition from quiescence to the proliferative state and traverse the various periods of the cell cycle leading to cell division. The analysis of gene function will be based on the results of DNA-RNA hybridization studies utilizing the nonrepeated sequences of DNA. We will also determine whether malignant cells are characterized by an altered pattern of gene transcription and, if so, whether this reflects the activation of normally unexpressed late-replicating genes.

BIBLIOGRAPHIC REFERENCES: Madore, H. Paul, and Bello, Leonard J., Stabilization of Adenovirus Nuclear RNA by Intercalating Drugs, Biochim. Biophys. Acta 520:404 (1978). Bilgin, Bilgen Arif, and Bello, Leonard J., Effect of Low Cell Density on Transition Probability, J. Cell Biology 79:8a (1978).

19. A MOLECULAR ANALYSIS OF DEVELOPMENTAL MUTATIONS

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The differentiation pathway a particular cell in a multicellular organism will follow is controlled by the specific pattern of gene expression in that cell. A model system, in which differential gene expression is observed, can be studied towards an eventual understanding of aberrancies in the pattern of gene expression leading to malignant behavior. The imaginal discs of the fly, Drosophila melanogaster, provide such a model system. The discs are small populations of cell set aside during larval development for later differentiation into adult structures. The bithorax genes of Drosophila are known to be essential to the imaginal disc cells in the transition from embryo to adult and have been extensively characterized genetically. This work proposes to isolate the bithorax genes and, using biochemical and cytological techniques, to characterize their expression at different stages of development. The many bithorax minus mutants will be studied to elucidate the cause of their aberrant gene expression.

In the course of this work, conditions will be established for two-dimensional DNA gels with resolution sufficient for use with higher eukaryotic genomes. Such a technique could facilate the isolation and study of, for example, the DNA fragment missing in leukemic patients carrying the Philadelphia chromosome.

20. GROWTH AND DIFFERENTIATED FUNCTION OF KERATINOCYTES

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The multiplication and differentiated behavior of the keratinocyte of stratified squamous epithelia will be examined in detail. Cultures of this cell type contain multiplying and terminally differentiating cells. The control of the transition of the cells from proliferative to terminally differentiating phases will be examined. The principal differentiated products of this cell type are the keratins and the cross-linked envelope. The synthesis of these products is regulated according to the stage of terminal differentiation, which is defined by the position of the cells in the natural epithelium or by the size of the cells. As the cells differentiate they change the keratins they synthesize, and begin the synthesis of new proteins (such as the envelope precursor) not synthesized in the cells of the basal layer. To a considerable extent, these changes also take place in cultured keratinocytes. The control of this process will be studed as a manifestation of controlled gene expression.

21. GENE ACTION AND CELLULAR DIFFERENTIATION IN CULTURE

Silagi, S., Graf, L. H., Calvelli, T. A., Cornell University, Medical Center, Cornell Medical College, Dept. of Obstetrics & Gynecology, *1300 York Ave., New York, New York*, 10021, United States of America

This work is aimed at exploring regulatory mechanisms involved in normal differentiation and malignant transformation, using the B16 melanoma model system involving melanotic and amelanotic malignant clones and amelanotic nonmalignant clones derived from the melanotic clone after growth and maintenance in the presence of 1 microgram/ml of 5-bromodeoxyuridine (BrdUrd). The BrdUrd clone can immunize syngeneic mice against the parental tumor. By using hyperimmune mouse antisera to the BrdUrd-grown clone and to irradiated parental malignant cells we are comparing glycoproteins and proteins of the 2 cell types by immunoprecipitation, immuodiffusion, immunofluorescence and cytotoxicity. Peritoneal exudate cells from immune animals can neutralize the tumorigenic ability of the malignant cells when coinjected into syngeneic mice. Macrophage:T cell cooperation appears to be involved. Delineation of the T cell type is under way. Peritoneal exudate cells from mice immunized with heat-killed BCG also neutralize malignant cells. The effector cells appear to be macrophages. We have shown that correlation between plasminogen activator (PA) activity and tumorigenicity and between inhibition of PA activity and tumorigenicity need not exist. We are examining the mechanism by which 5-azacytidine and other regulators of differentiated function operate. These approaches should lead to further understanding of regulatory mechanisms in malignancy, immuogenicity and melanogenesis.

22. REGULATION OF METABOLISM AND CELLULAR PROLIFERATION

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This is part of a broader project. A summary of this subproject is not available.

23. MOLECULAR BASIS OF UNCONTROLLED REPLICATION

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No summary has been provided to the Smithsonian Science Information Exchange.

2. NUCLEAR AND EXTRANUCLEAR PROTEINS (AND FACTORS) CONTROLLING DIFFERENTIATION AND PROLIFERATION

24. FUNCTION OF SPECIFIC NUCLEAR PROTEINS IN THE CONTROL OF CELL PROLIFERATION

O'Farrell, M. K., University of Essex, Dept. of Biology, *Wivenhoe Pk., Colchester CO4 3SQ, England, United Kingdom*

OBJECTIVE: The purpose of this investigation is to elucidate the role of two specific nuclear proteins in the control of proliferation of eukaryotic cells in culture.

APPROACH: It has been shown previously that serum stimulation of quiescent mouse 3T3 cells induces a general increase of 3-4 times in the rate of biosynthesis of nuclear proteins. Two nuclear proteins respond dramatically and specifically to growth stimulation: the synthesis of the second, P2, is coincident in time with the duplication of the chromatin. The polypeptide P2 will be purified and characterized. Antibodies will be raised to the polypeptide and the sub-nuclear localization examined. The function of P2 in nuclear physiology will be investigated by establishing in vitro systems for RNA transcription and DNA replication. The effect of addition of exogenous P2 or anti-P2 will be analyzed. We will also examine the importance of post-translational modification in this context. The problem of the function of these polypeptides will also be approached by examining their response to different types of restimulation, i.e., starvation and readdition of an amino acid and temperature shift of temperature-sensitive G1 mutants.

25. CELL DIFFERENTIATION AND CARCINOGENESIS - POSSIBLE CONTROL BY CONTROLLING TRANSCRIPTION NON-HISTONE PEPTIDE EFFECTOR

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The policy of our research program is based on the possible correlation between chromosomal peptides controlling transcription and the carcinogenesis process. The experiments are carried out by studying: (1) The level of peptides in the chromatin of experimental and human tumors as compared to homologous normal tissues. (2) The possibility that the chromosomal peptides are able to inhibit the carcinogenesis process especially at the promotion level.

The results obtained at this time show that the presence of controlling transcription peptides is markedly reduced in the chromatin of several experimental tumors (Novikoff heputoma, mouse fibrosarcoma, mouse lymphosarcoma, LP-59 fibrosarcoma cells 'in vitro').

Experiments are in progress to demonstrate the activity of chromosomal peptides controlling the RNA synthesis at transcriptional level in the chromatin of human leukemic leukocytes 'in vivo.'

26. CONTROLS OF CELL CYCLE AND CELL PROLIFERATION

Naha, P. M., Paterson Lab., Dept. of Cell Biology, *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: There are indications that differentiation and carcinogenesis are related to the regulatory changes in a cell which are possibly determined during G1 phase of the cell cycle. It has been suggested that there are some rate controlling signals in G1 itself and at the G1/S boundary which have regulatory function. Our objective is to decipher the controlling functions in G1.

APPROACH: Temperature-sensitive (ts) variant clones of BALB/c-3T3 were put on a linear time scale by measuring their time of entry into S phase (Nature, 258, 49-53, 1975). Complementation tests between the ts variants suggested (J. Cell Science, 35, 53-58, 1979) that there are at least three complementation groups, any of which could block a cell's entry into S phase. Initial biochemical studies suggested the presence of an additional non-histone protein in the ts variants blocked either early or late in the G1 phase (mol. wt. 24,000). This raised the possibility of a negative regulatory control of cell proliferation.

PROGRESS: Experimental conditions have been developed (J. Cell Science, in press) to induce G1 negative cells at high frequency by UV-irradiation of ts cells arrested in G1 phase at the restricted temperature. Neither the wild type nor the ts variants could be 'mutated' under similar conditions. Such G1 negative cells might be useful in the search for relevant 'repressor' protein controlling cell cycle proliferation.

27. ANALYSIS OF G1 IN MAMMALIAN CELLS

Baserga, R. L., McKircher, S., Temple University, School of Medicine, Dept. of Pathology, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

In eukaryotic cells, the critical steps for the regulation of cell proliferation apparently reside in the G1 phase of the cell cycle. Under conditions restrictive for growth, a number of cell strains and untransformed cell lines tend to arrest in a state of quiescence in G1, this resting stage often being referred to as G0. A number of temperature-sensitive (ts) mutant cell lines that arrest at the nonpermissive temperature, in a specific phase of the cell cycle, have been isolated. Two such ts mutant cell lines (AF8 and ts13, both derived from BHK cells) specifically arrest at the nonpermissive temperature in G1. When they are made quiescent by serum restriction and subsequently stimulated with 10% serum, these G1 ts mutants enter S phase at 34 degrees but fail to do so at 40 degrees. Similarly, polyoma infection of resting AF8 or ts13 cells causes these cells to enter S phase at 34 degrees but not at 40 degrees. However, infection with Adenovirus 2 causes these mutants to enter S phase, even at the nonpermissive temperature of 40 degrees. We propose a combination of G1 ts mutants and virus infection to identify proteins that regulate the cell cycle flow in critical G1 period. For this purpose, we shall use gel electrophoresis of immune complexes, identification of early viral mRNAs and microinjection into cells of DNA fragments produced by restriction endonucleases.

BIBLIOGRAPHIC REFERENCES: Rossini, M., G.J. Jonak and R. Baserga. Analysis of Adenovirus-Induced Cellular DNA Synthesis in a ts Mutant of the Cell Cycle. In: Animal Virus Genetics, ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XVIII, 1980, in press.

28. STUDY OF FACTORS CONTROLLING CELLULAR PROLIFERATION

Baserga, R. L., Waechter, D., Mercer, E., Temple University, School of Medicine, Dept. of Pathology, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

Under certain conditions non-permissive for growth, such as high density or serum deprivation, human or rodent fibroblasts enter a stationary phase, during which DNA synthesis and cell division all but cease. When these resting cultures are stimulated to proliferate (for instance, by serum), the cells enter, after a lag period, DNA synthesis and subsequently mitosis. We intend to continue our studies aimed at elucidating the intranuclear mechanisms controlling the flow of cells from the G0 to the S phase of the cell cycle, as well as the maintenance of transformation in culture. For this purpose, we shall use cell cycle-specific temperature-sensitive mutants, i.e., mammalian cells that grow normally at the permissive temperature (usually, 33 degrees or 34 degrees C) but arrest at a specific point of the cell cycle at the non-permissive temperature (39.5 degrees or 40 degrees C) We shall also use virally-transformed cells and study, by somatic cell hybridization, how they can regulate the cell cycle flow in G1-arrested temperature-sensitive mutants.

BIBLIOGRAPHIC REFERENCES: Soprano, K.J., Rossini, M., Croce, C. and Baserga, R. (1980) The Role of Large T Antigen in Simian Virus 40-Induced Reactivation of Silent

rRNA Genes in Human-Mouse Hybrid Cells. Virology 102, 317-326. Soprano, K.J. and Baserga, R. (1980) Reactivation of Ribosomal RNA Genes in Human-Mouse Hybrid Cells by 12-O-Tetradecanoylphorbol 13-Acetate. Proc. Natl. Acad. Sci. USA 77, 1566-1569.

29. MODULATION OF GENE EXPRESSION IN CELL CULTURE

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OBJECTIVES: 1) The ectopic production of polypeptide hormones by non-endocrine cells provides a model for analyzing the mechanisms responsible for gene expression. 2) The elucidation of simple chemical compounds capable of activating the expression of specific genes provides an approach to analyzing gene regulation. 3) Understanding of molecular events involved in gene activation provide insights into the control of specific protein synthesis with implications for normal differentiation and perhaps for the regression of normal function observed in neoplasia.

PLAN: To characterize the alterations and modifications of nuclear proteins that occur during gene activation to determine if protein-nucleic acid interactions are important in gene modulation.

METHOD: Separation and characterization of nuclear proteins both histones and nonhistone proteins from control HeLa cell cultures and replicate cultures grown with sodium butyrate and hydroxyurea (two compounds that activate glycoprotein hormone production in HeLa cells).

FINDINGS: Histone proteins have been successfully extracted from purified HeLa cell nuclei and separated according to change utilizing high-resolution polyacrylamide gel electrophoresis. Cells have been radioactively labelled in control, sodium butyrate-treated, and hydroxyurea-treated cultures prior to fractionation, and nuclear protein modifications have been observed by autoradiography of dried gels following histone separation.

CLINICAL RELATIONSHIP: This area of research is relevant to understanding alterations in gene activity associated with abnormalities in development and differentiation and perhaps to the process of neoplasia.

30. NUCLEAR VS EXTRANUCLEAR CONTROL OF GENE EXPRESSION

Moore, E. E., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Pathology, *4200 E. 9th Ave., Denver, Colorado,* 80262, United States of America

The long-term goal of my research is to understand the factor(s) determining the tumorigenic state of a cell, and the relationship of these factors to the regulatory mechanisms responsible for the development and maintenance of the differentiated state. The experiments described here are designed to determine the relative role of both nuclear and extranuclear factors in these regulatory events. Initially, two teratocarcinoma-derived cell lines will be used for this study; a) an embryonal carcinoma cell line (247-Des or PCC48azal) which is multipotenial and tumorigenic and b) a parietal yolk sac cell line (PYS-2) which expresses various 'differentiated' functions and is non-tumorigenic. Somatic cell hybrids will be prepared between these cell lines, and characterized for their tumorigenic and differentiated state. Cells will then be fractionated into a) karyoplasts, or nuclei surrounded by a small rim of cytoplasm and plasma membrane and b) cytoplasts, or enucleated cells. Various types of recombinant cells will be prepared and examined for their differentiated and tumorigenic state. The regulatory role of extranuclear factors will be investigated in cybrids, or recombinant cells formed by the fusion of cytoplasts with whole cells. Similarly, the role of the nucleus will be investigated by preparation and characterization of binucleated cells, or cells formed by the fusion of karyoplasts and whole cells. Lastly, various reconstituted cells will be prepared by the fusion of isolated karyoplasts and cytoplasts. Characterization of recombinant cells formed by all possible crosses, should provide insight into a) the complexity of the regulatory network, b) the nuclear vs. extranuclear location of the various regulatory factors and c) the nature of the interaction between the regulatory factors controlling the differentiated and tumorigenic state of the cell.

31. NONHISTONE CHROMOSOMAL PROTEINS AND HEPATOMA GROWTH

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The objectives of the program are to identify non-histone chromosomal proteins influencing tumor growth by using transplanted hepatomas as a model system. In this study, hepatomas showing a spectrum of growth rates will be used in order to correlate changes in non-histone chromosomal proteins with tumor growth rate. An attempt will be made to discriminate between transformation-linked and progression-linked alterations in hepatomas. Further purification of those proteins whose concentration has been shown in our preliminary studies to be changed in liver tumors will be performed. Particular attention will be directed to proteins subject to phosphorylation, and correlations between phosphate content and turnover and the rate of cell division will be investigated. In order to test the potential regulatory properties of isolated proteins, their influence on DNA structure and function will be examined. Structural changes will be followed in circular dichroism studies, and effects on function will be tested by action on DNA replication and transcription. The potential of proteins, which are either increased or decreased in amount in hepatomas, for exhibiting contrasting proterties in these test systems will be investigated.

32. HISTONES IN CELL DIFFERENTIATION AND CARCINOGENESIS

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We are investigating the origin and significance of drastic changes in the histone variant composition of nucleosomes during development, proliferation and differentiation of different cell types of the mouse, by studying: a) The molecular interactions of nonallelic primary structure variants and minor species of histones to form high affinity histone complexes and nucleosomes, b) the distribution of the various histone components in chromosome regions in different functional states, c) the molecular mechanisms of histone incorporation into chromatin in absence of DNA replication, in normal, regenerating and neoplastic liver cells as well as erythroleukemia cells before and after induction of terminal differentiation.

BIBLIOGRAPHIC REFERENCES: Franklin, S.G., Wolf, S.I., Zweidler, A. and Blumberg, B.S. Localization of the amino acid substitution site in a new variant of human serum albumin, albumin Mexico-2. Proc. Natl. Acad. Sci. USA 77, 2505-2509, 1980. Urban, M.K. and Zweidler, A. Changes in nucleosomal core histone variants during chicken development and maturation. Develop. Biol., 1981, in press.

33. CHROMATIN PROTEINS OF EMBRYONAL CARCINOMA CELLS

Oshima, R. G., Massachusetts Inst. of Technology, School of Science, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

Nuclear proteins associated with the differentiated cells formed by mouse embryonal carcinoma cells in vitro are identified, purified and characterized. Several mouse embryonal carcinoma cells are compared to extra-embryonic endodermal cell lines and freshly isolated endodermal cells. Changes in both histone and non-histome nuclear associated proteins are evident.

34. PROTEIN BINDING TO YEAST CENTROMERIC DNA

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The proposed project will be performed in Dr. John Carbon's laboratory where cloned genes from the yeast, S. cerevisiae, are being used to obtain centromeric DNA sequences by the overlap hybridization screening technique. We will attempt to isolate centromere specific DNA-binding proteins by 1) reconstitution of proteins and centomeric DNA in solution and subsequent dissociation of the bound proteins and centromeric DNA in solution and subsequent dissociation of the bound proteins, 2) DNA-cellulose chromatography, and 3) isolation of intracellular hybrid plasmid-protein complexes from transformed yeast cells followed by dissociation of the bound proteins. The

proteins will be characterized in terms of their DNA-binding properties by the nitrocellulose filter binding assay and for their molecular heterogeneity by gel electrophoresis. Centromere specific DNA-binding proteins will also be examined for their biochemical and immunological homology to microtubular proteins. The size and heterogeneity of the protein binding sites within centromeric DNA will be determined by restriction enzyme analysis of the protein-centromeric DNA complex. Characterization of the interaction of specific proteins with centromeric DNA should reveal their role in centromere structure and function and may provide fundamental principles governing the molecular mechanisms of cell division.

35. ENZYMATIC DNA MODIFICATION IN CELL DETERMINATION AND IN THE CONTROL OF NORMAL AND NEOPLASTIC GROWTH

Scarano, E., Tosi, L., Branno, M., de Franciscis, V., Maharajan, V., Terrana, B., Raia, C., Consiglio Nazionale delle Ricerche, Lab. of Molecular Embryology, *Via Toiano 2, Arco Felice, 80072 Naples, Italy*

OBJECTIVE: The aim of this project is to study whether nuclear enzymatic DNA modifications are related to oncogenic phenomena. This idea is based on the 'synchron model' which hypothesizes DNA modifying enzyme-caused heritable changes underlie ontogenesis and carcinogenesis, in higher eukaryotes. The synchron model predicts that enzymatic DNA modifications, some of them probably occurring via a two-step mechanism, methylation and aminohydrolysis of 5-methyl-cytosine at the DNA level, regulate the cell lineage of higher eukaryotes.

In the last two years several authors have reported data in agreement with some aspects of the synchron model. (Razin A. and Riggs A.D. (1980) - Science 210, 604-610.) Furthermore, heritable changes in the immunoglobulin genes have been reported to take place during the terminal differentiation of lymphocytes.

Environmental or genetic factors might cause similar events to occur in the differentiated cells of adult organisms. The appearance of specific DNA modifying enzyme might cause a normal cell to undergo heritable changes and become a cancer cell.

APPROACH: So far we have tested the effect of anticancer agents on the DNA methylase activity present during sea urchin and amphibian embryo early development. The state of methylation of specific genes may be studied with restriction endonucleases and this methodology will be used wherever possible.

BIBLIOGRAPHIC REFERENCES: Tosi, L. & Scarano, E. (1979). Macromolecules in the Functioning Cell (Eds. F. Salvatore, C. Marino & P. Volpe), Plenum Publishing Corp., New York, p. 239-253.

36. CONTROL OF DNA REPLICATION IN NORMAL AND TUMOR CELLS

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This proposal has as its objective an investigation of control of growth and DNA replication in normal and neoplastic tissues. Four major approaches will be used. First, we intend to purify and characterize DNA polymerases present in the nucleus. Activities as well as physical properties of the DNA polymerases from liver nuclei and hepatoma nuclei will be compared. Any differences observed between the two tissues will be investigated in a fast growing, an intermediate growing and a slow growing hepatoma. Secondly, we intend to identify, separate and, hopefully, reconstruct nuclear components that make up a replicating complex. It is by now obvious that factors other than DNA and DNA polymerase must be part of the replicating system. We will look for such factors in nuclei and investigate their possible significance in the control of DNA replication. Thirdly, we will use bleomycin to investigate possible differences in DNA repair in liver and hepatoma cells. Bleomycin stimulates 3H-TTP incorporation in a nuclear incorporating system. Stimulation of host liver nuclei is much more dramatic than stimulation of hepatoma nuclei. The possibility that this difference is due to a less efficient repair system in hepatomas will be explored. Fourthly, the effect of cytoplasmic or humoral factors on DNA replication will be investigated. There is evidence that such factors play a role in control of growth and DNA synthesis. By using factors from hepatoma tissues or tumor-bearing animals on nuclei or cells of liver, or factors from normal animals on nuclei or cells from hepatomas, we hope to obtain evidence that such factors might influence the loss of control in neoplastic cells.

BIBLIOGRAPHIC REFERENCES: A Difference in Bleomycin-Induced DNA Synthesis Between Liver Nuclei from Mature and Old Rats. Ove, P. and Coetzee, M.L. Mechanisms of Aging and Development. 8: 363 (1978). Selective Response of DNA Polymerase beta to Bleomycin-Induced Breaks in DNA. Coetzee, M.L., Chou, R., and Ove, P. Cancer Res. in press (1978).

37. PROTEIN METHYLATION IN NEOPLASTIC TISSUES

Paik, W. K., Kim, S., Kim, Y. S., DiMaria, P., Temple University, School of Medicine, Dept. of Biochemistry, *3322 Broad St., Philadelphia, Pennsylvania*, 19122, United States of America

The overall aim of the proposed research is to find possible causal relationship between the methylation of nuclear proteins and growth of tissues, with the following objective: (1) To investigate the possible relationship between the extent of methylation of nuclear proteins (histones and non-histone chromosomal proteins) and the degree of differentiation and rate of growth of neoplastic tissues. (2) To study the levels of various methylating and demethylating enzymes during liver regeneration and growth of neoplasms.

BIBLIOGRAPHIC REFERENCES: Studies on cytochrome c methylation in yeast. DiMaria, P., Polastro, E., DeLange, R. J., Kim, S. and Paik, W.K. J. Biol. Chem. 254, 4645 (1979). Methyl acceptors for protein methylase II from human erythrocyte membrane. Galletti, P., Paik, W.K. and Kim, S. Eur. J. Biochem., 97, 221 (1979).

38. RELATIONSHIP OF DIADENOSINE-5',5‴,P1,P4-TETRAPHOSPHATE (AP4A) TO GROWTH AND ITS REGULATION

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The unusual dincleotide diadenosine 5'5‴,P1,P4-tetraphosphate (Ap4A) was discovered by us in 1966, and was found to be present in a wide variety of living cells, prokaryotic and eukaryotic. Its concentration was approximately 10 to the minus 8th power M in resting cells, rising 100 fold to approximately 10 to the minus 6th power M in cells entering the S phase of the cell cycle. Ap4A has been found recently by Grummt and colleagues to associate specifically with a subunit of DNA polymerase alpha, and to induce replication eyes in resting cells, but no effect on cell-free DNA replication was observed.

It is proposed to prepare 3H-alpha 32P-Ap4A, and 3H-beta 32P-Ap4A, and to study their metabolic fate in a purified DNA initiation system catalyzed by DNA polymerase alpha.

3. TRANSCRIPTIONAL AND TRANSLATIONAL CONTROL OF DIFFERENTIATION AND PROLIFERATION

39. POSSIBLE MECHANISMS OF THE GENE ACTIVITY AT TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL LEVEL IN A EUKARYOTIC MODEL SYSTEM

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OBJECTIVE: The aim of the present research project is to gain basic information about control mechanisms in normal cells. The control of gene expression is of crucial importance for maintenance of the differentiated state of a cell, and disturbances in gene regulation may lead to dedifferentiation and conversion of normal cells to cancer cells. By investigation of the transcription process and the nature and processing of the products of transcription in a differentiated cell type, general, as well as specific, information about regulation mechanisms at transcriptional and post-transcriptional levels can be obtained.

APPROACH: The polytene salivary gland cells of the midge Chironomus tentans are used as a model system. The analyses of the transcriptional events are largely facilitated by the availability of a transcription inhibitor, that is, the purine

nucleoside analogue 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) as a probe. DRB acts selectively and reversibly on the synthesis of heterogeneous nuclear RNA in our, as well as in several other, cell systems by blocking chain initiation. Since exogenous adenosine also affects heterogeneous nuclear RNA and messenger RNA comparative electrophoretic analyses in gels and thin layer chromatographic analyses are designed in order to reveal the mechanism(s) by which DRB and adenosine interfere with the transcription mechanism. Post-transcriptional events are elucidated by investigation of events involved in post-transcriptional polyadenylation of heterogeneous nuclear RNA and messenger RNA, in particular, in the course of the biogenesis of two well-defined messenger RNAs (75 S and 35 S RNA).

40. CELL CYCLE CONTROL

Singer, R. A., Johnston, G. C., Easterbrook, K. B., Dalhousie University, Dept. of Biochemistry/Medicine, *Sir Charles Tupper Med. Bldg., Halifax, Nova Scotia,* B3H 4H7, Canada

OBJECTIVE: We have found that, for the yeast Saccharomyces cerevisiae, decreased production of ribosomal precursor RNA precedes G1 arrest when cells are subjected to any of the six treatments which cause cell cycle arrest. Our main objective is to investigate the mechanism which links transcription of rRNA genes with regulation of the cell cycle.

APPROACH: Our approach is to use specific restriction fragments of cloned yeast rDNA as probes for the presence of non-ribosomal portions of the ribosomal precursor RNA transcript, which may serve as enumerated 'coupons' of rRNA gene transcription. Subsequently we hope to identify the cellular machinery of this linkage by characterizing the RNA found in spindle pole bodies, the duplication of which is the first cell cycle event.

PROGRESS: Yeast ribosomal DNA, cloned in a standard E. coli plasmid vector, has been obtained, and restriciton fragments appropriate for detection of non-ribosomal portions of the ribosomal precursor RNA transcript have been generated. The four species of yeast rRNA, for use as competitor RNA in molecular hybridization studies, have been prepared and purified in quantity. Procedures for 32P-labelling of RNA, without extensive label incorporation into other material such as polyphosphates, and methods for further purification of labelled RNA, have both been worked out. Preliminary hybridization experiments using restriction fragments of ribosomal DNA immobilized on nitrocellulose paper show that RNA labelled in vivo can be purified sufficiently for further experiments.

41. CONTROL MECHANISMS IN HUMAN TUMOR CELLS - SMALL RNAS

Eliceiri, G. L., Rao, M. N., St. Louis University, School of Medicine, Dept. of Pathology, *1402 S. Grand Blvd., St. Louis, Missouri,* 63104, United States of America

We are studying some abundant, long-lived, homodisperse, low molecular weight nuclear RNA species of unknown function, particularly RNA species and D, in cultured human cervix carcinoma cells. C and D RNA exhibit several interesting characteristics: their transcription units may be up to 25 times larger than their known precursors; they seem to pass through the cytoplasm for a few minutes shortly after their transcription; and they appear to be present in heterogeneous nuclear RNA-protein particles. In addition, apparently about 5% of the cellular C and D RNA content is covalently linked to a low molecular weight subpopulation of chromatin DNA. Finally, RNA species C and D have 5'-end cap structures that are very similar to cap 2 of eukaryotic messenger RNAs, and D RNA markedly inhibits translation of mammalian messenger RNA in a cell-free extract.

Several types of experiments will be performed, toward the long-term objective of identifying the physiological role of these RNA species. First, through some in vivo experiments we wish to examine if D RNA might be involved in the inhibition of protein synthesis that occurs during mitosis. Another goal is to test if RNA species D and C are translated. A third aim is to study the dynamic relationship between the covalently-linked short DNA-small RNA complexes and a) the metaphase and interphase stages of chromatin, b) the known nucleoplasmic small RNAs, and c) bulk chromatin DNA. Finally, we wish to examine by microscopic autoradiography the intracellular behavior of purified labeled low molecular weight RNA species, after they have been microinjected into cultured human cells.

42. MESSENGER RNA METABOLISM IN MAMMALIAN CELLS

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The types of proteins and the efficiency with which they are made is often the essential determinant of phenotypic expression in cells and organisms. Changes in protein synthetic ability can be modulated either by altering the messenger RNA content or by changes in the efficiency of their translation. Since the overall objective of this research program is to understand the mechanism of mammalian cell-growth regulation, we sought to examine certain aspects of mRNA structure and function in quiescent and serum induced proliferating Balb 3T3 clone A31 cells.

The goals set for the current year were: to determine the mechanism of translational regulation in resting and serum induced 3T3 cells; secondly, to further characterize the protein associated with poly(A) regions in mRNA on the basis of its antigenic similarities; and thirdly, to focus on the structural features of a well characterized mRNA molecule in order to 'map' the protein binding sites.

43. RNA AND GROWTH CONTROL IN ANIMAL CELLS

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This project deals with the molecular details of messenger RNA biosynthesis -- the definition of transcription units in mammalian cells, the correspondence between these transcription units and mRNAs formed from them -- and the potential steps along this complicated pathway where regulation of mRNA supply is effected.

The use of recombinant DNA clones and specific segments of Adenovirus DNA has allowed the suggestion that regulation occurs both at the level of transcription and mRNA stabilization.

BIBLIOGRAPHIC REFERENCES: Weber, J., Blanchard, J.-M., Ginsberg, H., and Darnell, J.E. Jr. (1980). Order of polyadenylic acid addition and splicing events in early adenovirus mRNA formation. J. of Virol, 33, 286-291. Salditt-Georgieff, M., Harpold, M., Chen-Kiang, S., and Darnell, J. E. Jr. (1980). The addition of 5' cap structures occurs early in hnRNA synthesis and prematurely terminated molecules are capped. Cell 19, 69-78.

44. CONTROL OF DIFFERENTIATION IN CYANOBACTERIA

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OBJECTIVE: To determine the extent to which transcriptional control governs differentiation during heterocyst formation in filamentous blue-green algae.

APPROACH: To determine a transcriptional program during differentiation using two major approaches: a) Studies with antibiotics known to inhibit translation or transcription. b) Studies that quantitate the amount of newly synthesized RNA by measuring RNA:DNA hybridization. This includes construction of nif and other specific DNA probes.

OBJECTIVE: To determine what elements modulate transcription during heterocyst formation by selecting mutants which have altered phenotypes during differentiation.

CURRENT WORK: I am currently a predoctoral student in the Department of Microbiology at the University of Iowa. I work in the laboratory of Dr. Michael Feiss, where we study chromosome packaging in bacteriophage lambda.

45. CONTROL OF MYELOMA TUMOR GROWTH

Huang, R. C., Johns Hopkins University, School of Arts & Sciences, Dept. of Biology, *34th & Charles Sts., Baltimore, Maryland,* 21218, United States of America

Mouse myelomas have been chosen for use in the study of mechanisms involving the regulation of transcription and trans-

lation controls. Several cloned specific genes will be used as probes to determine how the transcriptional and translational activities of the tumors control the growth of the tumor.

BIBLIOGRAPHIC REFERENCES: Reeve, A. G. and Huang, R. C. A Method for the Enzymatic Synthesis and Purification of (alpha-32P)Nucleotide Triphosphates. Nucleic Acids Res. 6 (1) 81-91, 1979.

46. PROTEIN SYNTHESIS AND CONTROL OF THE CELL CYCLE

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Previous results have suggested that the rate of cell division in cultured cells is determined by the rate of accumulation of proteins required in the G1 phase of the cell cycle, with the rate of protein synthesis maintaining its active control function when the culture growth rate is altered. This hypothesis will be tested in human diploid fibroblasts, normal and malignant human epithelial cells, and two rapidly growing cell lines. The hypothesis implies a reciprocal-normal distribution of G1 phase times in continuously proliferating populations; and it predicts that when the culture growth rate is altered by a change in extracellular conditions, the new rate of movement through the G1 phase and the new rate of protein synthesis will be correlated. The hypothesis will be tested by determining (1) if a reduction in the culture growth rate is accomplished by reversible withdrawal of cells from the cell cycle, as would be expected from reciprocal-normal kinetics, (2) whether the G1 times of siblings are correlated, (3) what quantitative relationship is maintained between the rate of protein synthesis and the rate of passage through the G1 phase, and (4) if the kinetics of growth stimulation are consistent with the hypothesis.

BIBLIOGRAPHIC REFERENCES: Castor, LaRoy N. A cell cycle model based on variations in the inherited rate of passage through the G1 phase. J. Cell Biol. 83: 13a, 1979. Abstract.

47. CELLULAR MULTIPLICATION AND DIFFERENTIATION

Dove, W. F., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

This is part of a broader project. A summary of this subproject is not available.

48. NETWORKS OF DEVELOPMENTAL CONTROLS IN A MODEL SYSTEM

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This is part of a broader project. A summary of this subproject is not available.

49. REGULATORY MECHANISMS IN MAMMALIAN CELL PROLIFERATION

Datta, P., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Biological Chemistry, *1335 E. Catherine St., Ann Arbor, Michigan*, 48104, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

4. VIRAL CONTROL OF DIFFERENTIATION AND PROLIFERATION

50. CANCER RESEARCH PROGRAM - VIRUSES - CONTROL MECHANISMS

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This application is for continued general support of the research activities of the Department of Molecular Biology & Virus Laboratory. In July 1978 there will be fourteen active faculty members and one emeritus professor in this research group, together with about forty postdoctoral research associates, forty predoctoral students, and about one hundred people in technical and clerical capacities. All faculty members are supported in part by state funds and all have research grants that support their individual research programs which are housed in assigned core space in Stanley Hall. However, all of the faculty also use facilities and staff personnel housed in common use space and such facilities and services have for eighteen years been partly supported by a general program grant, most recently as a Cancer Center. Continued support of the Cancer Center is requested because we believe the total research in Stanley Hall is substantially related to the national cancer program, that a Cancer grant is economically and spacewise the most efficient way to support general facilities and services, and that facilities and services shared under this arrangement foster valuable exchanges among participating researchers.

The research in the Molecular Biology-Virus Laboratory Cancer Center includes such topics as the molecular biology of development employing Xenopus laevis and Drosophila melanogaster; gene regulation in eucaryotic cells (Tetrahymena) and procaryotic cells (E. coli and phages lambda and P2-P4); recombination mechanisms (E. coli); genetic structure and replication of DNA and RNA tumor viruses; structure and function of enzymes and other macromolecules; coordination of metabolism in normal and malignant cells; regulation of somatic cell growth and division; neuroembryological studies; mutagenesis and carcinogenesis.

51. LEUKEMIC CELL SYSTEMS - INDUCTION AND INHIBITION OF DIFFERENTIATION

Marks, P. A., Memorial Sloan Kettering Cancer Center, Division of Cell Biology, *1275 York Ave., New York, New York*, 10021, United States of America

OBJECTIVES: To define characteristics of the control of expression of differentiation in virus transformed cells, employing the murine erythroleukemia cell system infected by Friend virus complex. The present proposal outlines studies designed to define, at a cellular and molecular level, the expression of erythroid characteristics during induction of murine erythroleukemia cell (MELC) differentiation and the action of tumor promoters in inhibiting terminal differentiation. Specifically, we propose to investigate: 1. The expression of different markers of erythroid differentiation and the inhibition of this expression by tumor promoters, such as 12-0-tetradecanoyl-phorbol-13-acetate (TPA); 2. the effect of tumor promoters in inhibiting the expression of erythroid characteristics induced by different agents including hexamethylene bisacetamide (HMBA), dimethylsulfoxide (Me2SO), actinomycin-D (AMD) and hemin, which appear to have different primary cellular effects and lead to different patterns of expression of erythroid characteristics in MELC; 3. isolation and characterization of variant cell lines with altered sensitivity to inhibition of expression of differentiating characteristics when cultured with tumor promoters, as a tool for exploring the mechanism of action of tumor promoters; 4. the action of inhibitors of differentiation in relation to events in the cell division cycle; and 5. nuclear protein changes associated with differentiation and tumor promoter-mediated inhibition of differentiation.

The definition of the mechanisms controlling the expression of differentiated characteristics in MELC should provide basic information about the process of terminal differentiation in eukaryotic cells. These studies should also provide a better understanding of the actions of tumor promoters which may, in turn, provide a basis for developing a practical in vitro screening procedure to identify tumor promoters. Understanding gained in these studies will yield insights into mechanisms controlling normal cellular proliferation and differentiation. Such knowledge of normal growth control and differentiation and of the action of tumor promoters could influence our approaches to cancer prevention and treatment.

52. CONTROL MECHANISMS OF DIFFERENTIATION AND MALIGNANCY

Friend, C., Haddad, J., Tsuei, D., Pogo, B. G., Marovitz, W., Stringer, E., City University of New York, Mount Sinai School of Medicine, Dept. of Experimental Cell Biology, *1 Gustave L. Levy Pl., New York, New York*, 10029, United States of America

Our research program is concerned with the study of the pathogenesis of leukemia and the basic mechanisms involved in the suppression of the leukemic state. Our investigations into the murine virus (FLV)-induced erythroleukemia cell system continue. The cells can be induced to differentiate along the eryth-

roid pathway when grown in the presence of DMSO or a variety of other inducers.

Among the aspects to be studied in the differentiating cells are: 1) the mechanism of action of compounds which stimulate or inhibit differentiation; 2) the changes that occur in the cell membrane; 3) the modulation of gene expression; 4) DNA transcription and replication; and 5) cytoskeletal alterations.

These studies have been designed to provide insight into the mechanisms underlying the neoplastic state. Elucidation of the regulatory mechanisms involved in the shift from malignancy to differentiation may yield information which will aid in the development of new chemotherapeutic agents.

53. MOLECULAR AND PHYSIOLOGICAL CONTROL MECHANISMS IN NORMAL & NEOPLASTIC CELLS AND ORIGIN & PATHOGENESIS OF THE LEUKEMIAS & LYMPHOMAS

Gallo, R. C., Sarin, P. S., Breitman, T., Wong Staal, F., Saxinger, W. C., Barr, F., Bradley, E., Foote Reed, S., Smith, R. G., Gootenberg, J., Mier, J., Poiesz, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cellular Control Mechanisms Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

This Laboratory is concerned with six areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information to human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation in vitro. (This relates to a major interest of the laboratory: Does the phenotypic abnormality of leumemia in man result from a block in leukocyte maturation?) (5) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

BIBLIOGRAPHIC REFERENCES: Sarngadharan, M. G. and Gallo, R. C.: DNA Polymerases of Human Normal and Leukemic Cells. P. Mildner (Ed.): FEBS Federation of European Biochemical Societies, Enzyme Regulation and Mechanism of Action. England, Pergamon Press, 1980, pp. 145-158. Collins, S. J., Bodner, A., Ting, R. and Gallo, R. C.: Induction of Morphological and Functional Differentiation of Human Promyelocytic Leukemia Cells (HL60) by Compounds which Induce Differentiation of Murine Leukemic Cells. Int. J. Cancer 25:213-218, 1980.

54. PROGRAM IN DEVELOPMENTAL BIOLOGY OF CANCER

Pierce, G. B., Erikson, R. L., Lehman, J. M., Nakane, P. K., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Pathology, *4200 E. 9th Ave., Denver, Colorado,* 80262, United States of America

It has been shown through the study of three kinds of carcinoma that the progeny of malignant stem cells have the capacity for differentiation, yielding benign and, in one case, normal progeny. Direction of this naturally occurring differentiation of tumors could serve as an alternative to cytotoxic therapy. To this end, a multidisciplinary approach to the study of differentiation in tumors will be mounted. It will include input from experimental pathologists, cell biologists, molecular biologists, biochemists, and developmental biologists. Focus will be on three essential processes: the environment of differentiating cells, the plasma cell membrane which must mediate environmental signals, and finally, the way neoplastic genomes are controlled.

Specifically, we will study control of genomes through the study of the synthesis of the transforming protein encoded for in the src region of RNA tumor viruses. Membrane studies will include molecular studies of transbridging proteins and comparisons of the metabolisms of normal and neoplastic membranes including the distribution and turnover of glycoproteins, and effects of growth promoters on membranes. Comparisons will be made of asymmetrical distribution of proteins of membranes of karyoplasts and nucleoplasts of normal and malignant cells to obtain information on cell-cell interactions.

Comparison will be made of the effects of DNA tumor viruses on differentiation of cells or early embryos and of teratocarcinomas. Flow microfluorimetry will be done on temperature-sensitive mutants of SV40 to determine the mechanism of action. The role of embryonic fields in directing differentiation of neoplastic cells will be studied. Functional differentiation in developing pituitary glands, pituitary tumors, and in liver cells will be studied.

BIBLIOGRAPHIC REFERENCES: Miller, G.J. and Pierce, G.B. Induction of foam cell morphology in aortic medial cells by phorbol-12-myristate-13-acetate (PMA). Fed. Proc. 39:429, 1980. Pierce, G.B. Teratocarcinoma. In: Cancer Markers: Developmental and Diagnostic Significance (Stewart Sell, ed.), Humana Press, Inc., Clifton, N.J., 1980, pp. 1-36.

55. CANCER CENTER SUPPORT (CORE) GRANT

Eckhart, W., Salk Inst. for Biological Studies, *10010 N. Torry Pines Blvd., P.O. Box 1809, La Jolla, California,* 92037, United States of America

The objective of our current program is to understand the mechanisms of growth control of normal and malignant cells. The program is organized in four parts: Tumor virology: These studies are aimed at understanding the genetic organization of RNA and DNA tumor viruses and the effects of viral gene expression of cell growth regulation. The objective is to define the nature and control of molecules that affect cell growth regulation. 2. Cell surface immunology and biochemistry: We are using immunological, biochemical and genetic techniques to study the nature, function and regulation of selected molecules on normal and malignant lymphocytes. These studies are aimed at understanding how surface molecules in general, and tumor-associated molecules in particular, are controlled, and at understanding the properties of cells of the immune system whose function it is to recognize and kill tumor cells. 3. Mammary differentiation: We are studying the differentiation of the mammary gland in rats and mice, the growth control of mammary epithelial cells, mechanisms of carcinogenesis and the properties of mammary cancer cells. The objective is to develop approaches toward prevention and treatment of breast cancer. 4. Gene expression: We are studying the genomic organization and control of expression of genes expressed in normal and malignant cells. The objective is to develop approaches toward controlling the growth of tumor cells.

56. NORMAL AND VIRUS TRANSFORMED CELLS - MRNA BIOGENESIS AND GENETIC REGULATION

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This research is concerned primarily with nucleic acid metabolism in mammalian cells featuring studies on the biogenesis of messenger RNA. Growing cells not transformed by viruses as well as cells transformed by and infected by DNA tumor viruses are being studied with the major effort devoted to the details involved in the transcription and conversion of large nuclear RNA molecules into functional polysomal messenger RNA. The long-range goal is to understand the regulation of mRNA production in mammalian cells since this must be a pivotal point of regulation in cell growth.

57. LEUKEMIA CELL SYSTEMS - INDUCTION OF DIFFERENTIATION

Marks, P. A., Rifkind, R. A., Bank, A., Reuben, R., Columbia University, College of Physicians & Surgeons, Dept. of Medicine, *630 W. 168th St., New York, New York,* 10032, United States of America

The overall objectives of this research program continue to be the investigation of the regulation of the coordinated expression of nonlinked genes during the course of differentiation of eukaryotic cells. The system employed in these studies has been the Friend virus complex infected murine erythroleukemia cell (MELC).

BIBLIOGRAPHIC REFERENCES: Yamasaki, H., Weinstein, I.B., Fibach, E., Rifkind, R.A. and Marks, P.A. Tumor promoter-induced adhesion of DS19 clone of murine erythroleukemia cells. Cancer Res. 39: 1989-1994, 1979. Fibach, E., Gam-

bari, R., Shaw, P.A., Maniatis, G., Reuben, R.C., Sassa, S., Rifkind, R.A. and Marks, P.A. Tumor promoter-mediated inhibition of cell differentiation: Suppression of the expression of erythroid functions in murine erytholeukemia cells. Proc. Natl. Acad. Sci. (US). 76:1906-1910, 1979.

B. CELL SURFACE COMPONENTS AND MEMBRANE FUNCTION IN DIFFERENTIATION AND GROWTH

1. ROLE OF CELL SURFACE ANTIGENS IN DIFFERENTIATION AND PROLIFERATION

58. CELL SURFACE ANTIGENS INVOLVED IN DROSOPHILA DEVELOPMENT

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We propose to study the cell surface antigens on the imaginal disks of Drosophila melanogaster. Imaginal disks are packets of cells located in the larva which differentiate during metamorphosis into adult cuticular structures. Prior to metamorphosis groups of cells within the disk become committed to particular paths of development. The pattern of this commitment has been studied in great detail. Of particular interest is the observation that boundaries are established which separate groups of cells destined to form different parts of the adult.

An obvious hypothesis is that these boundaries are initiated and maintained by changes in cell surface components. By producing a set of monoclonal antibodies specific to disk cell surface antigens, we can use indirect immunofluorescence to determine the distribution of given determinants within a disk. Antigens whose distribution respect the developmental boundaries will be further studied for their possible roles in determination.

59. CELL SURFACE AND GROWTH CONTROL

Smets, L. A., Collard, J. G., Temmink, J. H., Nederlands Kankerinstituut, Cancer Research Lab., *108 Sarphatistraat, Antoni van Leeuwenhoek Lab., Amsterdam, Netherlands*

OBJECTIVE: To study the surface changes in transformed and malignant cells in relation to defective growth control. Emphasis is laid on a study of the specific agglutination of tumor cells by plant lectins.

APPROACH: Correlation between antigen expression and Con A agglutination of leukemic cells. In a transplantable mouse lymphosarcoma, a correlation between antigen expression and Con A agglutinability has been observed. Animal and human leukemic cells acquire increased agglutinability by tissue culture, and the mouse cells thus modified are a better vaccine against untreated tumor cells than cells inactivated by conventional methods.

Agglutination with Con A as a probe for antigenicity and the effect of tissue culture on antigen expression are currently studied for their value in active immunotherapy.

Detection and distribution of lectin binding sites on the surface of normal and transformed cells: The increased agglutination of transformed versus normal cells has been studied in 3T3 cells at the ultrastructural level. Detection of lectin binding sites by various cytochemical methods has been compared with binding of labeled lectins. Moreover, scanning and transmission electron microscopic studies of cells growing on a substrate or suspended for the agglutination assay have revealed important differences in cell surface morphology. As a result, it could be demonstrated that the actual density of binding sites was about 10-fold higher on transformed than on normal cells, although both cell lines bound about equal amounts of labeled Con A.

60. THY-1 DIFFERENTIATION ANTIGEN IN RAT MAMMARY TISSUE

Ritter, M., Pain, C., Rudland, P. S., Imperial Cancer Research Fund, Membrane Immunology Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

We have demonstrated the presence of Thy-1 antigen on in vivo rat mammary tissue, using immunofluorescence on wax-embedded tissue sections. Thy-1 is found on ductal and alveolar myoepithelial cells of normal, pregnant and lactating animals (myoepithelial cells identified by Coomassie blue staining of myofilaments and by their position between the epithelial cells and the basement membrane). Thy-1 positive myoepithelial cells were also found in Dimethylbenzathracene and N-methylnitrosourea-induced mammary adenocarcinomas, and in adenocarcinomas derived from Rama 25 cells (cuboidal epithelial stem cell line).

Rat mammary cell lines were also studied by immunofluorescence. Rama 25, a stem cell line, was Thy-1 negative, whilst its myoepithelial derivative (Rama 29) was Thy-1 positive. Thy-1 antigen was also present on the elongated cells that appeared as fresh converts within several stem cell line cultures (Rama 75, 401 and 402). Clonal cell lines intermediate in morphology between the stem cell cuboidal line Rama 25 and the elongated myoepithelial-like cells of Rama 29 have been isolated. These intermediate types convert to myoepithelial-like cells when cultured at high frequency; during this conversion the appearance of surface Thy-1 correlates with the main morphological change and with the appearance of the extracellular matrix protein, fibronectin. Whether Thy-1 plays a causative role in the conversion process remains to be established.

61. NORMAL AND NEOPLASTIC DEVELOPMENT OF THE MAMMARY GLAND

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The analysis of cell types in rat mammary gland based on their growth response to the phases of the estrus cycle will be completed. An analysis of lesions induced by nitrosomethylurea in the rat mammary gland will be carried out, and the cell types involved will be determined. The specificity of monoclonal antibodies prepared to rat and human mammary cells will be studied, in terms both of the cell types to which they bind in vivo and of the cell surface proteins they immunoprecipitate in cultures. New monoclonal antibodies to human mammary cells, both normal and neoplastic, will be isolated. The role of the Thy-1 antigen in the differentiation of rat mammary cells will be studied further. The mechanism by which lipids (fatty acids, lysolecithins) induce dome formation in cultures of rat mammary cells will be pursued.

BIBLIOGRAPHIC REFERENCES: R. Dulbecco, M. Bologna, and M. Unger. Differentiation of a rat mammary cell line in vitro. Proc. Natl. Acad. Sci. USA, 76, 1256-1260 (1979). R. Dulbecco, M. Bologna, and M. Unger. Role of Thy-1 antigen in the in vitro differentiation of a rat mammary cell line. Proc. Natl. Acad. Sci. USA, 76, 1848-1852 (1979).

62. DEVELOPMENTAL AND ANTIGENIC PROPERTIES OF MOUSE TERATOCARCINOMA CELLS

Illmensee, K., Bluthmann, H., Cicurel, L., van Tuinen, E., Universite de Geneve, Dept. of Animal Biology, *Rue de Candolle 3, 1211 Geneva 4, Switzerland*

OBJECTIVE: The aim of our research is to find surface antigens characteristic for a particular teratocarcinoma cell line. These antigens may represent powerful ontogenic markers for the study of cell lineage and differentiation. In addition, the detection of teratocarcinoma or tumor-specific antigens is pursued in order to determine the cell surface changes involved in malignant transformation.

APPROACH: We are using the hybridoma technique for the detection of surface antigens. Mouse or rat spleen cells, previously immunized with teratocarcinoma cells, were fused with mouse myeloma cells and the resulting hybrid cells grown in culture. Those cells secreting antibodies of interest were then cloned and expanded in order to yield high amounts of a specific monoclonal antibody. We are using the enzyme-linked immunosorbent assay (ELISA) for the screening of our hybridoma supernatants.

PROGRESS: Only these hybridomas that showed a positive and exclusive reaction with the tumor cells were finally retained. Several of these hybridoma clones were then subcloned and their secreted antibodies analyzed on viable tumor cells by immunofluorescent staining techniques. We are currently extending our tests for cellular and developmental specificity of the monoclonal antibodies using embryonic and adult tissues as target cell material.

63. CELL INTERACTIONS IN TETRATOCARCINOMA DIFFERENTIATION

Rosenstraus, M. J., Rutgers the State University of New Jersey, New Brunswick Campus, Douglass College, Dept. of Biological Sciences, *New Brunswick, New Jersey*, 08903, United States of America

The role of cell-cell interactions and stage specific embryonic antigens in early mammalian embryogenesis will be studied using clonal murine embryonal carcinoma (EC) cell lines as a model system. The time at which EC cells in aggregates become irreversibly committed to differentiate into endoderm will be determined by constructing bilayered aggregates consisting of a core of EC cells surrounded by a rind of genetically marked exogenous endoderm cells; in the presence of the exogenous endoderm, endodermal differentiation of EC cells is prevented. To study the developmental functions of embryonic cell surface antigens, variant cell lines with altered cell surfaces will be isolated and characterized. Lectins and antibody (monoclonal SSEA-1) plus complement will be used to select the variants. Variant cell lines will be tested for their ability to differentiate in vitro and during tumor formation in vivo. The cell surface changes responsible for resistance to the cytotoxic effect of the lectins will be analyzed initially by separating labeled glycoproteins by gel electrophoresis. Finally, to help define cell functions required for EC cell differentiation, EC cell variants with altered developmental potential ('nullipotent' cells) will be analyzed by somatic cell hybridization. Complementation tests will be performed to determine if different 'nullipotent' lines possess a common defect.

BIBLIOGRAPHIC REFERENCES: Rosenstraus, M.J., Balint, R.B. and Levine, A.J. (1980) Pluripotency of somatic cell hybrids between nullipotent and pluripotent embryonal carcinoma cells. Somatic Cell Genetics 6:555-565. Rosentraus, M.J. and Levine, A.J. (1979) Alterations in the developmental potential of embryonal carcinoma cells in mixed aggregates of nullipotent and pluripotent cells. Cell 17:337-346.

64. ONCOFETAL ANTIGENS IN MOUSE EMBRYOGENESIS AND TUMOR GROWTH

Rosenberg, S. O., University of Maryland, Baltimore County Campus, Graduate School, Dept. of Biological Sciences, *Catonsville, Maryland*, 21228, United States of America

The objectives of this research are to detect and characterize embryonic and oncofetal antigens of various embryonic systems and a tumor system, and to assess the involvement of these antigens in pre-implantation mouse embryogenesis and tumor growth. Immunological techniques for antigen recognition and functions are being employed and include: (1) Mixed lymphocyte reactions, (2) cell-mediated lympholysis, (3) lymphocyte-induced growth inhibition, (4) antibody-induced growth inhibition, (5) cross-reactivity of serologically defined and lymphocyte defined antigens, and (6) scanning electron microscope localization of defined antigens on preimplantation mouse embryos. These experiments should further our understanding of the role of oncofetal antigens in preimplantation mouse development and tumor growth.

65. ROLE OF CELL SURFACE ANTIGENS IN MAMMALIAN DEVELOPMENT

Solter, D., Wistar Inst. of Anatomy & Biology, *36th & Spruce Sts., Philadelphia, Pennsylvania*, 19104, United States of America

The basic aim of the proposed research is to characterize stage-specific surface proteins (SSSP) and to examine their role during early development and differentiation of mouse embryo. Syngeneic immunization with embryos of different developmental stages will be used to obtain specific antisera. Surface proteins of preimplantation mouse embryos will be labeled, immunoprecipitated with specific antisera and characterized on one- and two-dimensional gels. Spleen cells from immunized mice will be fused with myeloma cells and hybrids producing monoclonal antibodies against stage-specific surface proteins isolated. Teratocarcinoma stem cell lines will be tested with anti-embryo sera and those with cross-reacting antigens detected. Using monoclonal antibodies and teratocarcinoma stem cells, a sufficient amount of SSSP will be isolated and characterized in detail. Anti-embryo sera will be used to probe the properties of the embryonic cell membrane and to assess the role of SSSP in differentiation and development of embryos and teratocarcinoma stem cells in vitro.

BIBLIOGRAPHIC REFERENCES: Howe, C.C., Gmur, R. and Solter, D.: Cytoplasmic and nuclear protein synthesis during in vitro differentiation of murine ICM and embryonal carcinoma cells, Develop. Biol., 74: 351-363, 1980. Levine, G., Ballou, B., Reiland, J., Solter, D., Gumerman, L. and Hakala, T.: Localization of 131I labeled tumor specific monoclonal antibody in the tumor bearing BALB/c mouse. J. Nucl. Med., 21: 570-573, 1980.

66. CONSOLIDATED BASIC CANCER RESEARCH PROGRAM

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The CORE Program is designed to encompass the cancer research activities of The Wistar Institute. The key objectives of the program concern: 1) modulation of the phenotypic expression of cellular antigens in normal and malignant cells; 2) components of the immune response and their interaction in the host-tumor response; 3) structural changes in cell surface membranes in normal and transformed cells; 4) virus-cell interaction in neoplastic transformation - determination of some regulatory events involved in this interaction; 5) the role of chemical carcinogens in oncogenesis; 6) regulatory elements associated with cell growth and the phenotypic expression of specific cell functions; 7) application of quantitative cytochemistry and cell cycle analysis to tumor biology; and 8) genetic analysis of regulatory aspects of oncogenesis. In pursuit of these objectives, a wide variety of biochemical, biophysical and biological methods will be employed.

BIBLIOGRAPHIC REFERENCES: Herlyn, D., Herlyn, M., Steplewski, Z. and Koprowski, H. 1979. Monoclonal antibodies in cell-mediated cytotoxicity against human melanoma and colorectal carcinoma. Eur. J. Immunol. 9:657-659. Herlyn, D., Steplewski, Z., Herlyn, M. and Koprowski, H. 1980. Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. Cancer Res. 40:717-721.

67. CELLULAR BIOLOGY OF MALIGNANT TRANSFORMATION

Rieber, M., Rieber, N. S., Alonso, M., Instituto Venezolano de Investigaciones Cientificas, Centro de Microbiologia y Biologia Celular, Laboratorio Bioquimica Celular, *Aptdo. 1827, Carretera Panamericana Km. 11, Caracas 1010A, Venezuela*

OBJECTIVES: 1) To find markers for differentiation of epithelial cells during the progressive stages of conversion to a malignant phenotype. 2) To find markers for differentiation of tumoral cells with varying metastatic potential.

METHOD: 1) Biochemical study of the cell surface and cytoskeletal macromolecules. 2) Study of the interaction of the cell with the extracellular matrix. (Translated)

68. IMMUNOCHEMICAL ANALYSIS OF MYOBLAST DIFFERENTIATION

Kaufman, S. J., University of Illinois, Urbana Champaign Campus, School of Liberal Arts & Sciences, Dept. of Microbiology, *Urbana, Illinois*, 61801, United States of America

OBJECTIVE: To define the antigenic changes on the cell surface of developing myoblasts and to identify which of these components are germane to the differentiation and fusion of these cells. Comparisons of the membranes of the normal myogenic cells with developmentally defective transformed and tumorigenic mutants will be made.

APPROACH: Monoclonal antibodies directed against cell surface determinants will be prepared and used to isolate and quantify antigenic membrane components present on the differentiating myoblast and on their transformed counterparts.

PROGRESS: Monoclonal antibodies specific for different stages of development have been isolated. These antibodies detect alterations in composition of the membranes of the normal and the transformed cells.

2. ROLE OF CELL SURFACE CARBOHYDRATES IN DIFFERENTIATION AND PROLIFERATION

69. PURIFICATION OF PLASMA MEMBRANES FROM PRE-IMPLANTATION MOUSE EMBRYOS

MacQueen, H., Imperial Cancer Research Fund, Cell Biology Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

It is widely believed that the cell surface plays a vital role in the development of the mammalian embryo. However, no specific function in embryonic cell interaction or morphogenesis has yet been ascribed to a surface component. It is our aim to follow changes in surface proteins and glycoproteins during preimplantation development of the mouse embryo, and to examine the relationship between these changes and cell differentiation. Our approach was to collect mouse embryos at the two-cell stage and to culture them in vitro, radio-labeling them at different stages by addition of 35S-methionine to the medium before isolating the plasma membranes. The amount of starting material is very small, but losses can be minimized by the addition of non-radioactive teratocarcinoma cells as carrier. The two-phase separation method of Brunette and Till (Journal of Membrane Biology, 1971, 5, 215) has been recommended for small-scale preparations, but we have found that the sucrose step-gradient method of Pilkis et al. (Biochemica et Biophysica Acta, 1974, 343, 250), with certain modifications to include protease inhibitors throughout the procedure, yields better results. Purity of the final membrane preparation was judged by electron microscopy appearance and by differential enzyme activity. Comparison by SDS-PAGE and auto-fluorography of membranes from embryos labelled at different stages has revealed several stage-specific membrane proteins. These will be further characterized and it is hoped that one or more can be shown to play a role in the developmental process.

70. STRUCTURE, BIOSYNTHESIS AND ROLE IN DIFFERENTIATION OF MEMBRANE GLYCOPROTEINS

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The long-range goal of this project is the structural and functional characterization of cell membrane glycoproteins. Previous results from many laboratories including ours have demonstrated that plasma membrane preparations from many different sources show multiple glycopeptide bands when examined by SDS-gel electrophoresis. An understanding of the chemical nature of individual proteins will allow us to define structural, antigenic, and genetic relationships among these proteins. It will be of help to understand the significance of multiple glycoprotein components in these membrane systems. It will furthermore provide the sound basis for studies on the assembly of membranes during biosynthesis, on post-translational modifications, and their role for differentiation and on structural changes which may occur in the transformed state. The overall goal of this project will be approached by extending previous observations through a series of specific problems: I. Define more precisely the chemical and immunochemical nature of the different sialoglycoproteins in human erythrocyte membranes. II. Isolate and characterize sialoglycoproteins from red blood cells of mouse, sheep, and horse erythrocyte membranes. III. Develop procedures and analyze membrane glycoproteins from nucleated cells in various tissues. IV. Define more precisely the conformation and nature of the interaction of glycophorin A subunits. V. Investigate the biosynthesis of red cell membrane glycoproteins in an in vitro system. VI. Study the sialoglycoproteins in a differentiating system.

71. STRUCTURE AND ROLE OF A TRANSFORMATION-SENSITIVE CELL SURFACE GLYCOPROTEIN

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The major cell surface glycoprotein fibronectin is decreased after malignant transformation and is involved in cellular adhesion. We have investigated its mechanism of action. Polypeptide structural domains containing specific binding sites for collagen and the plasma membrane have been isolated and characterized biologically. High-affinity, separate binding sites for hyaluoronic acid and heparin were also characterized, and a third domain for heparin binding was identified.

The role of fibronectin in normal differentiation was examined. Cartilage cell development involves the loss of cell surface fibronectin, apparently due to a loss of receptors. Vitamin A reverses these events. Maintaining fibonectin levels inhibits cartilage cell development.

Our objectives will be to determine the composition and structure of the multiple active sites of fibronectin, and the mechanisms by which this major cell surface protein helps to regulate cell behavior and differentiation.

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72. PURIFICATION OF MEMBRANE ASSOCIATED REGULATORS OF CELL GROWTH AND DIFFERENTIATION

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OBJECTIVE: To characterize proteins and glycoproteins essential for cell growth and differentiation.

APPROACH: 1) Characterization of in vitro 'regulators' of human hemopoietic progenitors of granulocytes and T-lymphocytes. 2) Characterization of comparative in vitro regulators of mouse hemopoietic progenitors of granulocytes and T-lymphocytes and the 'help' factors for induction of T-lymphocyte cytotoxic response to specific antigen or replacement of macrophages for generation of antibody-producing cells in vitro. 3) Use of anti-human beta2-microglobulin (beta2m) affinity chromatography to prepare large quantities of purified growth factors from mouse and human conditioned medium for biological and chemical characterization. 4) Use of fluorescence polarization cell sorting for detection of target populations for growth factors.

PROGRESS: 1) One step purification by affinity chromatography for both human and mouse growth factors with preservation of bioactivity has been proven feasible. 2) Multiple step purification and affinity chromatography provide milligram quantities of factors suitable for more detailed structural analyses. 3) Fluorescence polarization cell sorting based on hemopoietic progenitors' response to growth factors has demonstrated some hemopoietic lineage specificity.

73. LYMPHOCYTE ANTIGENS - STRUCTURE, FUNCTION AND SYNTHESIS

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The objective of the research project is to identify and characterize surface glycoproteins of mouse lymphocytes with the long-term goal of understanding lymphocyte differentiation and function. Monoclonal antibodies have been obtained against several mouse lymphoid tissue-specific surface glycoproteins including Thy-1 and T200 glycoproteins. These reagents are being used to study various aspects of hematopoietic differentiation in the mouse and to investigate the biosynthesis of specific cell surface molecules. Efforts to obtain monoclonal antibodies which can be used to biochemically detect surface glycoproteins restricted to specific subpopulations of mouse lymphocytes will continue. Such antibodies will be characterized on a panel of mouse hematopoietic cell lines and selected antibodies then used in genetic, biochemical and functional studies of mouse lymphocyte cell surfaces.

BIBLIOGRAPHIC REFERENCES: Hyman, R.,I.S. Trowbridge, and K. Cunningham. Cell surface molecules of Friend erythroleukemias: Decrease in T200 glycoprotein expression after induction. J. Cell. Physiol., in press, (1981). Hyman, R., and I.S. Trowbridge. Two complementation classes of T200

(Ly5) glycoprotein-negative mutants. Immunogenetics, in press, (1981).

74. STUDIES ON THE ROLE OF CELL SURFACE CARBOHYDRATES IN THE DIFFERENTIATION OF DICTYOSTELIUM DISCOIDEUM

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OBJECTIVES: To study the role of cell surface carbohydrates in cell-cell interaction in Dictyostelium discoideum.

APPROACH: The changes in cell surface carbohydrates during the establishment of cell-cell contacts during the differentiation of Dictyostelium discoideum will be assessed by biochemical and immunological analyses of purified plasma membrane preparations. In addition, plasma membranes will be fractionated and the isolated components assessed for their ability to block 'in vitro' cell adhesion. Finally, attempts will be made to identify the molecules that induce spore and stalk cell differentiation.

75. THE GOLGI COMPLEX IN CELL REGULATION - CONTROL OF EXPRESSION OF CELL MEMBRANE CONSTITUENTS

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The cell surface and more specifically, integral membrane glycoproteins and glycolipids are implicated in the processes of increased growth and transformation as well as metastasis.

Fundamental studies over the past few years have documented a regulatory step at the level of the Golgi complex in the insertion and retrieval of integral membrane proteins to and from the cell surface. This proposal aims at genetically dissecting out the structural-functional correlates of saccules and vesicles in the Golgi complex by studying the biogenesis of several mutants of vasicular stomatitis virus in various cultured cells. The object is to attempt to provide a morphologic-genetic map of regulation at the level of the Golgi complex.

The studies will be applied to membrane proteins of oncoviruses and other integral membrane glycoproteins correlated with tumorigenicity. It is hoped the fundamental knowledge accrued from proposed studies can be eventually turned to therapeutic advantage.

76. ROLE OF GLYCOPROTEINS IN SEA URCHIN DEVELOPMENT

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Normal embryonic development and morphogenesis require highly regulated cellular interactions involving intercellular adhesion. Cell surface complex carbohydrates and their receptors are thought to specify such contacts. The proposed project investigates the role of glycoproteins in changes in cell adhesive properties during morphogenetic events of the sea urchin embryo, especially during cellular ingression of the primary mesenchyme. Major changes in glycoproteins will be detected with electrophoretic and specific visualization techniques. Cell surface glycoproteins will be identified by labeling intact cells with the galactose oxidase and lactoperoxidase methods. Direct electrophoretic analysis of the glycoproteins of isolated and cultured cells of the sixteen cell embryo, as well as analysis with conjugated lectins, will be used to determine whether specific glycoproteins are unique to various populations of cells. The effect of monospecific antibody on cell adhesion and ingression will be used to determine if a specific cell surface glycoprotein is involved in such phenomena. Experiments are also proposed to distinguish among various levels at which cells modulate the expression of both glycoproteins and cellular affinities at their surfaces.

77. CELL SURFACE ALTERATIONS IN MALIGNANCY

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This grant project proposes to study rat intestinal epithelial cell glycosyltransferases and cell membrane glycoproteins in order to determine their role in the control of differentiation. Methods have been established which separate the differentiated villus cell from the undifferentiated crypt cell. Furthermore, intestinal plasma membranes have been purified and separated from a purified Golgi fraction. We propose to utilize these techniques to (1) analyze and characterize the changes in intestinal epithelial cell surface membrane glycoproteins associated with cell turnover, differentiation and tumor induction, (2) characterize the changes in intestinal epithelial cell surface membrane glycoprotein synthesis associated with differentiation and malignant transformation.

We will also study animal tumor models we have recently described, where growth of the tumors correlated with the appearance and levels in serum of an electrophoretically distinct peak of galactosyltransferase on polyacrylamide gels (galactosyltransferase isoenzyme II). These animal tumor models will be studied in order to determine the role of cell membrane glycoproteins and tumor glycosyltransferases in tumor growth and metastases. We propose specifically to (1) characterize the biological properties of the tumor-associated serum galactosyltransferase isoenzyme II, and (2) purify and characterize the tumor galactosyltransferases and compare them with normal tissue and the serum isoenzymes. It is hoped that these studies will further define the cell membrane changes peculiar to tumor growth and the ability of a tumor cell to metastasize.

BIBLIOGRAPHIC REFERENCES: Quaroni, A., Kirsch, K., and Weiser, M.M.: Synthesis of Membrane Glycoproteins in Rat Small-Intestinal Villus Cells. Redistribution of L-(1,5,6-3H) Fucose-Labelled Membrane Glycoproteins Among Golgi, Lateral Basal and Microvillus Membranes In Vivo. Biochem. J. 182:203-212, 1979. Podolsky, D.K., and Weiser, M.M.: Purification of Galactosyltransferase 'Isoenzymes' I and II. J. Biol. Chem. 254:3983-3990, 1979.

78. CELL SURFACE CHANGES IN EPIDERMAL DIFFERENTIATION

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The epidermis is an excellent model system for the study of differentiation and carcinogenesis. Epidermal differentiation is a genetically programmed sequence of morphological and functional events in which there is a rapid turnover of distinct cell populations. In other tissues, alterations in the cell membranes have been found to be associated with stages of differentiation and with neoplasia; it has been further postulated that cell membranes may play a key role in the regulation of differentiation.

Separated populations of epidermal cells will be reacted with specific surface labels including lectins, in order to characterize the major glycoproteins present on the external surface of the membrane of intact viable cells. Membrane preparations will be electrophoresed on SDS-polyacrylamide gels and reacted with radiolabelled lectins; the glycoproteins will then be isolated by affinity chromatography on lectin columns. Purified glycoproteins will be injected into rabbits to produce antibodies. These procedures will be carried out for normal cell populations at different stages of differentiation and with basal cell carcinoma and squamous cell carcinoma. For a particular population, radioimmunoassays with the specific antibody and 125I labelled antigen will assess the extent of cell surface shedding. Radioimmunoassays will also be used to measure the cross-reactivity of antibodies and antigens drawn from different cell populations, normal and neoplastic. The overall aim is to characterize surface glycoproteins, and to explore distinctions among glycoproteins, from normal cell populations at various stages of differentiation and from carcinomas, that might serve as markers of these populations.

79. ANALYSIS OF EUCARYOTIC CELL SURFACES

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The hypothesis being tested is that the initiation and maintenance of growth control is a function of intercellular adhesion, probably affected by macromolecular events at the level of the cell membrane. These phenomena must involve the interaction of specific molecules that trigger further changes on the surface of the participating cells. Since transformed cells have a lower

level of tissue organization and altered growth and adhesive properties, these differences would be reflected in altered membrane architecture and/or molecular fine structure. Transformed and normal cells show such differences with respect to complex carbohydrates (glycoproteins and glycolipids). In some instances complex carbohydrates have been implicated as playing a role in growth regulation and in intercellular adheshion which ultimately governs morphogenesis and metastasis.

The proposed research plan is a direct approach to isolate compounds from the surfaces and members of synchronized cells that have the ability to influence growth and adhesion, and to determine the biochemical event involved in those interactions.

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80. GLYCOPROTEIN SYNTHESIS AND FUNCTION DURING THE CELL CYCLE

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Strong correlations exist between changes in the compositional and physical properties of the cell surface and the proliferative activity of cells. Reports dealing with normal cells (at mitosis and stimulated to proliferate by various means), transformed cells and differentiating cells support this conclusion and indicate that alterations in cell surface carbohydrate structures, probably in the form of glycoproteins, are instrumental in mediating these changes in proliferative behavior. Differences in surface carbohydrate structure have been demonstrated between growing and non-growing cells. Virally transformed cells also appear to exhibit their major diffcrences in surface protein structure at the level of their carbohydrate constituents. Finally, the only biochemically defined cell division cycle mutant in mammalian cells manifests its lesion at the stage of protein glycosylation. Nonetheles:s, the precise molecular alterations involved in these situations remain to be defined.

It is the thrust of this proposal to investigate the relationship between surface expression of specific carbohydrate types and the cell division process. The two approaches employed will be: 1) stimulation of cell division followed by rigorous analyses of the glycoproteins that subsequently are synthesized at various cell cycle stages; 2) specific inhibition of protein glycosylation followed by anlyses of the susbsequent ability of the cells to proliferate.

3. ROLE OF MEMBRANE FLUIDITY AND IONIC HOMEOSTASIS IN DIFFERENTIATION AND PROLIFERATION

81. HYDROGENATION, MEMBRANE FLUIDITY AND CELL REPLICATION

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OBJECTIVE: Cell membrane fluidity is important for determining many features of the cell, e.g., the flow of molecules into and out of the cell and also the movement of molecules such as proteins within the plane of the membrane. This fluidity is determined by the degree of unsaturation of the lipids of the membrane as well as the cholesterol content. The aim of the project is to manipulate the fluidity of cell membranes by a) hydrogenating the constituent double bonds and b) changing the cholesterol content and to determine how these factors can alter cell replication processes.

APPROACH: A water-soluble derivative of the homogeneous catalyst, a triphenyl phosphine chlororhodium complex, is being employed to hydrogenate (under conditions of high pressure) the unsaturated lipids of model and biological membranes. The reactions are monitored by analysis of the constituent fatty acids, by gas liquid chromatography. Studies are also in progress to manipulate the cholesterol content of cell surface membranes.

PROGRESS: Satisfactory hydrogenation of some simple membrane systems has been achieved. Studies are in progress to determine the effects of hydrogenation on membrane integrity.

Liposomes have been used to modulate the cholesterol content of lymphocyte membranes and produce characteristic and reproducible changes in membrane structure as seen by freeze-fracture electron microscopy, and in Na-K transport across the cell membranes of viable lymphocytes. The Na-K pump is responsible for maintaining cellular homeostasis.

82. MEMBRANE MICROVISCOSITY IN RELATION TO GROWTH CONTROL OF MURINE FIBROBLASTS

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OBJECTIVE: To investigate the role of cell membrane lipid fluidity in density-dependent inhibition of growth and escape from growth control after transformation. To determine the changes in cell membrane lipid microviscosity after transition of non-proliferating cells (G0 cells) into cycling cells and during the different phases of the cell cycle.

APPROACH: With the help of the fluorescence polarization technique, using 1,6 diphenyl 1,3,5 hexatriene as a probe, changes in membrane lipid fluidity are investigated after serum stimulation of contact-inhibited 3T3 cells. The distribution of cells in the cell cycle phases is measured by impulse cytophotometric determination of DNA per cell. In addition, similar studies are performed in synchronized cultures. With these techniques, the membrane lipid microviscosity will be studied in relation with density dependent inhibition of growth and loss of growth control. Furthermore, by modifying the membrane lipid fluidity, the possible role of membrane lipid fluidity in growth control will be investigated.

83. MECHANISMS OF LIPID PEROXIDATION IN CANCER CELLS, AND RELATIONSHIP OF PEROXIDATION AND ALDEHYDIC PRODUCTS TO CONTROL OF CELL DIVISION

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OBJECTIVES: To investigate the reasons underlying the low rate of lipid peroxidation in the endoplasmic reticulum of many tumor cell lines in comparison to normal tissues; to study the importance of lipid peroxidation, and the associated production of aldehydes, in relation to the control of cell division.

APPROACH: Microsomal fractions will be prepared from normal and tumor tissues and lipid peroxidation will be stimulated by the addition of: (a) NADPH, (b) NADPH/ADP/Fe ion and (c) ascorbate. The progress of lipid peroxidation will be monitored by measuring malonaldehyde production, oxygen consumption, and the utilization of polyunsaturated fatty acids.

The inhibitory actions of tumor microsomes on peroxidation of normal microsomes will be studied by mixed tissue incubations.

The effects of aldehydes (produced by lipid peroxidation) on DNA-synthesis and on membrane fluidity will be studied using radioactive tracer techniques and nitroxy-spin label ESR spectroscopy progress.

PROGRESS: Lipid peroxidation has been found to be depressed in several tumor tissues so far examined. No evidence of an increased anti-oxidant activity in tumor microsomes has been found. Lipid peroxidation has been found to be associated with a decreased membrane fluidity.

84. ENERGY METABOLISM, BIOLOGICAL OXIDATION AND CANCER GROWTH CONTROL

Galeotti, T., Cittadini, A., Bartoli, G. M., Dani, A., Bassi, D., Wolf, F., Scuderi, F., Palombini, G., Festuccia, G., Calviello, G., Universita Cattolica del Sacro Cuore, Inst. of General Pathology, *Via Pineta Sacchetti, 644, 00168 Rome, Italy*

OBJECTIVES: (1) The aim of the present project is to establish a correlation between the limited amount of lipid peroxides produced by membranes (microsomes, mitochondria) of experimental tumors and the loss of control of cell proliferation. (2) Study of Ca(plus plus) metabolism in cancer cells and evaluation of the role of Ca(plus plus) in the pathogenesis of biochemical (glycolysis and Pasteur effect) as well as biological (uncontrolled proliferation) characteristics of cancer cells.

APPROACH: (1) We have approached problem (1) by a) testing the degree of lipid peroxide production in hepatomas with different growth rates; b) analyzing the chemical composition and physical organization (order and motion parameters) of membrane phospholipids. (2) Study of Ca(plus plus) movement and fluxes across cancer cell plasma membrane both in vivo and in vitro. Comparison between cancer (Ehrlich ascites on thymoma cell) and normal cells, possibly of the same nature and origin (normal thymus cells). Modification of cell energy metabolism as a consequence of Ca(plus plus) movement and content in the cell.

PROGRESS: (1) The results indicate an inverse relationship between lipoperoxides and mitotic activity with the most perturbated patterns associated with the membranes of the maximal-deviation tumors. (2) The results so far obtained indicate that cancer cell plasma membrane can be considered highly and freely permeable to external Ca(plus plus) and that an increased Ca(plus plus) influx determines in normal cells metabolic characteristics peculiar to the neoplastic cell.

85. MEMBRANE DYNAMICS IN NORMAL AND TUMOR CELLS

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Benzo-pyrene-transformed 3T3 (BP-3T3) cells can be arrested in the G1-phase of their cell cycle by a 100-fold reduction in the serum concentration of the growth medium. Under such conditions alpha-aminoiso-butyric acid uptake is reduced 3-fold. However, the uptake of uridine and certain glucose analogs is not reduced. This is in contrast to 'normal' 3T3 cells which exhibit decreased transport rates for these same compounds. This suggests that hexose sugar and uridine uptake changes occurring during G1-arrest of 3T3 cells may be G0-specific events, or that the G1-arrest states in normal and transformed cells have different characteristics.

The H6-15 subline of SV-3T3 cells is a temperature-sensitive derivative which demonstrates normal growth behavior at 39 degrees C, but transformed behavior at 32 degrees C. 3-0-methylglucose transport is 2-4-fold higher when the cells are grown at 32 degrees C. However, at both the restrictive and permissive temperatures there is a marked cell density-dependent reduction in uptake. This seems inconsistent with hexose sugar transport activity being a determinant of growth potential of transformed cells. In addition, preliminary experiments indicate a 3-4-fold difference in serum component-depletion activity when cells are grown at 32 degrees C. This is consistent with the hypothesis that transformed cells have reduced serum requirements for growth.

86. IONIC INITIATION OF FIBROBLAST PROLIFERATION

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Pathobiologic considerations suggest that autonomous cell proliferation is the essence of neoplasia. We have already demonstrated that normal chicken fibroblasts are maintained in a quiescent state, while Rous sarcoma virus-infected fibroblasts proliferate actively, in a culture medium of reduced calcium and magnesium concentration. On the basis of these findngs we have hypothesized that calcium and magnesium have a role in the initiation of fibroblast replication and that failure of cytosolic divalent cation homeostasis may contribute to the autonomous initiation of replication of sarcomatous fibroblasts. We now propose to: (1) Extend our studies to include the effects of reduction of culture medium sodium and potassium, as well as calcium and magnesium, on the proliferative behavior of normal and RSV-infected fibroblasts. In order to test our hypothesis that ions initiate the G0-to-G1 transition, the effect of each ion will be studied by the technique of flow cytometry. (2) Study plasma membrane integrity ('extracellular marker' exclusion), membrane potentials, cation entry rates and nominal cation contents of normal and RSV-infected fibroblasts, with a view towards further testing of our hypothesis and identification of the defect in RSV-infected cells that might be responsible for failed cytosolic cation homeostasis. (3) Determine whether the following substances will initiate the proliferation of normal fibroblasts in reduced cation medium: serum vs. plasma, Cytochalasin B, ionophores, cyclic nucelotides, serotonin and the plant growth hormones auxin and cytokinin, phorbol myristate acetate. (4) Determine whether serine protease inhibitors (leupeptin, antipain, etc.), fibronectin or inhibitors of oxidative phosphorylation are capable of abolishing the autonomous proliferation of RSV-infected fibroblasts in reduced cation medium, without affecting the appropriate proliferation of normal fibroblasts in physiologic cation medium.

87. REGULATION OF THE CELL CYCLE AND DIFFERENTIATION OF NEUROBLASTOMA CELLS IN VITRO - ROLE OF ION TRANSPORT

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OBJECTIVE: To reveal the molecular mechanisms underlying the regulation of growth and differentiation of neuroblastoma cells, in particular the possible role of the plasma membrane ion transport properties.

APPROACH: The intrinsic modulations in cation transport properties, including the plasma membrane-bound (Na plus-K plus)-ATPase, are studied by tracer-flux methods and biochemical techniques. The possible role of these membrane properties in the regulation of the cell cycle and differentiation is established directly by modification of the cation transport properties, and indirectly by experimental modification of the plasma membrane composition.

88. SODIUM ION FLUXES AND MITOGENIC SIGNALING

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It is not known how any mitogenic peptide or tumor promoter activates a quiescent cell. In 1978, we reported evidence that certain peptide growth factors act by rapidly increasing Na permeability of the quiescent cell. Transformed cells in culture apparently contain more Na and/or are more permeable to Na than non-transformed ones. The greater permeability to Na probably causes the increase in Na-K pump activity which occurs soon after mitogenic activation and, at least in part, the greater pump activity in some transformed cell lines. Recently we reported that the enhancement of Na-dependent amino acid transport, which is also highly correlated with the activation of the quiescent cell in vitro, may in turn be due to the hyperpolarizing effect of pump stimulation since the pump is electrogenic. We plan to further explore the hypothesis that an increase in cell permeability to Na plays an important role in transformation, tumor promotion, and mitogenic signal transduction. Secondary changes in free Ca or protons may be responsible for altering energy metabolism and macromolecular syntheses. We call this hypothesis stimulus-division coupling since the proposed alterations in cation transport are analogous to the ones involved in muscle contraction and secretion.

The activity of the Na specific and non-specific cation transporters will be assayed as a function of cell density and growth state in non-transformed and transformed fibroblasts, by following both Li and 22Na ion entry and exit. Effects of internal and external Ca and MG ions, purified plasma membranes, individual peptide mitogens and tumor promoters, e.g., vasopressin and phorbol esters, will be examined. Amiloride-derivatives will be tried as irreversible inhibitors and photo-affinity labels of the amiloride-sensitive Na transporter. Growth of fibroblastic and two mammary carcinoma cell lines, in completely defined media, will be used to look for specific antagonists of the mitogenic response to vasopressin, phorbol esters and other tumor promoters. Na transport inhibitors, retinoids, selenium, Li and Rb ions will be tried. The mechanism and regulation of Na and Ca transport will be studied in the MCF-7 and ZR-75-1 lines of mammary carcinoma cells and in purified membrane vesicles from fibroblastic cells.

89. MECHANISMS OF MEMBRANE FUNCTION IN CELL GROWTH

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This application seeks support for a research program directed toward a better understanding of the role of the plasma membrane in the regulation of growth in cultured cells. The specific objective of the proposed investigation is to examine the relationship between four membrane-associated, physiological parameters (cell membrane potential, Ca plus 2 influx, cell cAMP levels, and nutrient transport) which are altered upon mitogen stimulation of normal cells and upon cell transformation. The experiments will seek the solution to four major unsolved problems: First, are changes in these four membrane parameters causally interrelated or are they altered independently? Second, does mitogen-stimulation or viral transformation of cells induce a change in one of these parameters which then triggers changes in the other three parameters? Third, does the binding of purified mitogens stimulate changes in these parameters by directly influencing membrane function or indirectly by stimulating changes in cellular metabolism which act to modify membrane function? Fourth, do internally produced growth factors in virally transformed cells influence membrane function by acting through mitogen receptors?

Experiments designed to answer these questions involve measurements of fluxes of tracer molecules across cellular membranes and membrane vesicles, and measurement of cAMP concentrations and membrane potentials in quiescent and mitogen-stimulated human fibroblasts and in other mammalian cells transformed by a temperature-sensitive Rous sarcoma virus.

90. REGULATION OF CELL GROWTH BY MEMBRANE ION FLUX

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Increased flux of Na and K ions across the cell membrane with a resultant increase in intracellular K ion concentration has been proposed to play a critical role in the regulation of cell proliferation (Rozengurt and Heppel, 1975). The present study will demonstrate if this increased ion flux is a necessary prerequisite for replication of Balb/c-3T3 cells. In addition, regulation of ion flux by the cell growth stimulating factors from serum, platelet-derived growth factor (PDGF), and somatomedin, as well as by the tumor promotor tetradecanyol phorbol acetate (TPA) and by the oncogenic virus SV40 will be explored. The effects of these agents on ion flux will be studied both in whole cells and in membrane preparations and membrane vesicles from cells arrested at several defined 'points' in the G0/Gl phase of the cell cycle. Systematic analysis of direct effects of these agents on ion flux in membrane vesicles from the growth-arrested cells will define the roles of Na-K ions ATPase and other ion transport mechanisms in the regulation of cell growth.

91. FUNCTION AND GROWTH OF A KIDNEY EPITHELIAL CELL LINE

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Our efforts over the past year have been aimed at defining the differentiated and growth properties of the distal tubular kidney epithelial cell line, MDCK. We plan to continue to concentrate our effort on these two problems. We are characterizing two Na ions transport systems in these cells: one is a H ions/Na ions antiport system which is inhibited by amiloride and is energy independent; the other is an Na/K ions cotransport system which is furosemide inhibited and appears to be ATP dependent. We are also attempting to ascertain the roles of cAMP, Na ions transport and rates of nutrient transport in the regulation of cell growth.

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92. ARCHITECTURE AND DYNAMICS OF HEPATOCYTE PLASMA MEMBRANES

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The long-term objective of this research proposal is to investigate the structural and functional alterations in hepatocyte plasma membranes associated with the neoplastic state. The program is concerned with the surface architecture of normal and neoplastic hepatocytes, as well as the dynamic properties of the plasma membranes. Studies will also be concerned with the mechanism of Ca2 ion binding to normal and neoplastic hepatocyte plasma membranes and its role in the regulation of membrane structure and function. By investigating these various phenomena one can further elucidate the role of plasma membranes in the regulation of cell growth in normal and neoplastic cells.

93. MODIFICATION OF TUMOR CELLS AND TUMOR SYSTEMS

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Our aim is to determine whether the rate of growth of non-tumor cells is limited or controlled, under physiological conditions, by the levels of intracellular monovalent cations or the activity of the Na/K ATPase pump system. We would also like to find out if tumor cells have escaped from such a regulatory system.

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94. ROLE OF IRON IN THE CONTROL OF NORMAL AND MALIGNANT CELL GROWTH

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One of the major problems confronting the molecular biologist is the understanding of the molecular events that control growth of mammalian cells. In general, the multiplication of cultured untransformed mammalian cells requires serum and in defined media (media without serum) it requires the presence of the serum iron carrier glycoprotein transferrin (Tr). Many transformed cells have lower serum requirements than the untransformed counterparts and some which proliferate in defined media do not require Tr. From these considerations, it can be suggested that some transformed cells must be able to regulate iron transport by a mechanism different from Tr mediated iron uptake. Thus, the understanding of differences and similarities in the mechanisms of iron transport between normal and transformed cells may be of great significance for the understanding of transformation. In order to study this problem two major complementary lines of work are in progress: 1) the elucidation of the interaction of Tr with specific receptor, particularly in normal cells, and its relation to cell proliferation; 2) identification of ways in which transformed cells transport iron in the absence of Tr.

95. CALCIUM AND GROWTH REGULATION IN NORMAL AND MALIGNANT CELLS

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No summary has been provided to the Smithsonian Science Information Exchange.

96. EFFECT OF PH ON CELLULAR GROWTH AND FUNCTION (HUMAN, MICE, HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

97. ROLE OF DISULFIDES IN NORMAL AND TUMOR CELL GROWTH

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Many diverse observations suggest that extracellular, as well as intracellular, thiol-disulfide reactions are associated with cell growth control and that viral cell transformation results in changes in such phenomena. A major hindrance to further investigations of the importance of such observations is the lack of a quantitative, sensitive, and specific analytical method for analysis of low molecular weight thiol-disulfide components. The specific aims of the present studies are: (1) to develop a general method for analysis of thiols, and thereby disulfide components, based upon isolation of thiols by affinity to a Sepharose-linked mercurial, conversion of the thiols to mixed disulfides of cystine, and quantitative measurement of the latter by conventional amino acid analysis; (2) to utilize this methodology to analyze the thiol, disulfide, and protein mixed disulfide content of two lines of normal cells (3T3 and MDCK), of two corresponding lines of virally transformed cells (SV40-3T3 and MSV-MDCK), and, simultaneously, of the growth media during exponential growth at low cell density and during growth at high cell density (confluent cells).

BIBLIOGRAPHIC REFERENCES: Fahey, R.C., G.L. Newton, R. Dorian, and E.M. Kosower (1980) Fed. Proc. 39 1701. Fahey, R.C., G.L. Newton, R. Dorian, and E.M. Kosower (1980) Anal. Biochem. 107 1.

98. ROLE OF WATER IN CANCER CELL DIVISION

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The objective of this research is to determine the role of water in cancer cell division by investigating the changes in the physical state of water that occur during the synchronized HeLa cell cycle. Cells will be grown and synchronized by established cell biology methods. Samples taken during mitosis, G1, S, and G2 will be examined by nuclear magnetic resonance spectroscopy (NMR). Spin-lattice, T1, and spin-spin, T2, relaxation times of intracellular water hydrogen protons will be determined at 30 MHz and 25 degrees C by pulsed NMR techniques. Spin echo techniques will be used to determine the self diffusion coefficient of water (D) in cellular protoplasm. Percent water content and electrolytes will be determined through the cell cycle by gravimetric analysis and flame photometry.

Changes in the physical state of water in the cancer cell during the cell cycle, as defined by the NMR parameters, will be correlated with the known biochemical events of the cell cycle. Methodology is proposed to study the contributions of chromatin, microtubules, and actin fibers to the cellular structure of water by macromolecular manipulation.

The state of water in karyoplasts and cytoplasts of each phase of the cycle, prepared by the Cytochalasin B enucleation technique, will be examined by NMR and the relative contribution of each to the state of cellular water will be determined.

These experiments will allow an evaluation of the water-macromolecular interactions characteristic of the HeLa cell cycle and will provide insights into the role that water plays in the events leading to cell division.

BIBLIOGRAPHIC REFERENCES: P.T. Beall, B. Szopa, S.R. Burzynski, and C.F. Hazlewood; Polypeptides that inhibit human breast cancer cell division. Cancer. Biochem. Biophys., 1978, in press. P.T. Beall, D. Medina, P.K. Seitz, M. Olive, and C.F. Hazlewood; The 'systemic effect' of elevated tissue and serum relaxation times for water in animal and human cancers. In: NMR in Medicine, Pacific Press, 1978, in press.

4. ROLE OF CELL SURFACE ENZYMES, RECEPTORS AND OTHER ARCHITECTURAL COMPONENTS IN DIFFERENTIATION AND PROLIFERATION

99. MEMBRANE STRUCTURE AND ENZYME INDUCTION

Chen, K. Y., Rutgers the State University of New Jersey, New Brunswick Campus, Douglass College, Dept. of Chemistry, *New Brunswick, New Jersey*, 08903, United States of America

The principal objective of this proposal is to study some of the biochemical processes that underlie differentiation; particular emphasis will be placed on the involvement of plasma membrane and ornithine decarboxylase (ODC). Mouse neuroblastoma cells can be induced to differentiate by cAMP. The differentiated cells lose their tumorigenicity. Increased tyrosine hydroxylase (TH) activity and neurite outgrowth are two distinct phenotypic expressions of differentiation in neuroblastoma cells. The possible involvement of membrane protein phosphorylation in mediating the effect of cAMP in cytodifferentiation will be studied. Differences in the composition of plasma membrane proteins of malignant and differentiated neuroblastoma cells will be probed by two surface labeling techniques (lactoperoxidase catalyzed iodination, and galactose oxidase catalyzed reductive tritiation), as well as by phosphorylation of purified membranes catalyzed by endogenous protein kinases. cAMP causes the induction of ODC and TH in neuroblastoma cells. These two enzymes are the rate-limiting enzymes in the synthesis of polyamines and catecholamines, respectively. The mechanism by which cAMP induces ODC, and the involvement of ODC in cytodifferentiation will be studied. The approach is to delineate the relationship between cAMP-induced membrane protein phsophorylation changes and the increase in ODC activity. The temporal and/or causal relationship between the cAMP-induced increase in ODC activity and TH activity will be investigated. The approach is to correlate changes of the two enzyme activities under conditions where ODC activity is varied. The proposed studies should help us to understand the role of cAMP and polyamines in cytodifferentiation. In addition, knowledge on the composition of plasma membranes of malignant vs. differentiated cells should help us to gain a better insight into the understanding of tumor reversion. Our long-term goal is to be able to design drugs which specifically modulate the various biochemical processes which underlie differentiation, so that differentiation and tumor reversion can be manipulated pharmacologically.

100. SURFACE CHARACTER OF TRANSFORMED CELLS

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Cells in culture from tumor origins exhibit loss of density-dependent inhibition of growth, (relative to non-tumor cells). Similar behavior is found among virally transformed populations, and this altered character is accompanied by modulation of the expression of cell surface sialoproteins and phosphoesterases. HeLa cells, e.g., regain a measure of normal growth inhibition when exposed to glucocorticoids. The modulation of sialoprotein and phosphoesterase activity during the response to glucocorticoid further implicates these activities in crucial cellular responses to population density.

The aim of our studies is to elucidate the role of these surface entities in the control over proliferation. A surface-replica cytochemical technique (SRC), developed here, permitted the localization of both sialic acid residues and alkaline phosphatase activity over the surface of HeLa cells; confirmation of a doubling of surface sialic acid in responses to dexamethasone; and demonstration of a cell-growth-cycle-dependent redistribution of both of these functional expressions. Studies of synchronously dividing HeLa cells suggested that the primary effect of glucocorticoids is prolongation of a portion of the G1 stage in growth. With extramural support these studies have been expanded to include quantitative (EDX) analysis of the expression of sialyltransferase, as well as the activities described, over both endothelial cells and HeLa cells-grown synchronously with the aid of nocodazole--in replica and/or thin section. Preliminary results support our hypothesis that surface sialyltransferase activity is stimulated by contact between microvilli from non-daughter cell pairs, while their growth is arrested.

BIBLIOGRAPHIC REFERENCES: Fiskin, A. M., Melnykovych, G. and Peterson, G.: Transient expression of lanthanum binding sites over surfaces of HeLa cells. Eur. J. Cell Biol. 21:151-159, 1980. Fiskin, A. M., Melnykovych, G. and Peterson, G.: Transient expression of alkaline phosphatase over HeLa cell surfaces. Eur. J. Biol. 21:160-166, 1980.

101. ROLE OF CELL SURFACE IN INITIATION OF CELL DIVISION

Cunningham, D. D., Low, D. A., Rofer, C., University of California, Irvine Campus, California College of Medicine, Dept. of Microbiology, *Irvine, California*, 92664, United States of America

We plan to study the mechanisms by which glucocorticoids and proteolytic enzymes such as trypsin and thrombin initiate cell division. Our studies on glucocorticoids will be directed at examining the changes in specific growth factor binding that occur after treatment with dexamethasone, a synthetic glucocorticoid. We plan to further study the relationship between the modulation of growth factor binding by dex and the modulation of the action of these growth factors by dex. Our efforts will be directed at the question of whether the modulations of growth factor binding are causally involved in the modulation of growth factor action. Our studies on the mechanisms by which proteolytic enzymes initiate cell division will be based on our findings that for both thrombin and trypsin, action at the cell surface is sufficient to initiate cell proliferation. We now plan to look for cell surface proteolytic cleavages that are causally involved in this initiation. These studies will employ cell surface labeling by the 125I-lactoperoxidase technique followed by one or two-dimensional electrophoresis and autoradiography.

BIBLIOGRAPHIC REFERENCES: Carney, Darrell H., Kevin C. Glenn, and Dennis D. Cunningham, 1979. Photoaffinity Labeling of a Single Receptor for alpha-Thrombin on Mouse Embryo Cells. J. Biol. Chem. 254:6244-6247. Simmer, Robert L., Joffre B. Baker, and Dennis D. Cunningham. 1980. Direct Linkage of Thrombin to Its Cell Surface Receptors in Different Cell Types. J. Supramol. Struc., in press.

102. MEMBRANE PATHOLOGY IN CARCINOGENESIS

Scott, R. E., Boman, B. M., Mayo Foundation, Section of Experimental Pathology, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

Significant changes in the cyclic AMP-dependent phosphorylation of specific plasma membrane proteins associated with malignant transformation have recently been identified in my laboratory and we have shown that aberrant activity of an endogenous plasma membrane cyclic AMP-dependent protein kinase is responsible for these changes. We have also identified differences in the cyclic AMP-dependent phosphorylation of plasma membrane proteins in differentiated and undifferentiated cells and have shown that modulation of cyclic AMP-dependent plasma membrane protein phosphorylation occurs during the G1 phase of the cell cycle in non-transformed cells. Studies proposed in this grant are designed to test the hypothesis that specific plasma membrane phosphoproteins influence control of cell growth in nontransformed cells but not in transformed cells. Experiments will be performed to characterize the endogenous cyclic AMP-dependent phosphorylation system in a variety of cell systems and to identify the biochemical basis for the defect in the phosphorylation system in transformed cells; both the protein kinase and its plasma membrane substrate will be studied. We will also examine the mechanisms for regulation of this phosphorylation system and we will attempt to identify the function(s) of the plasma membrane phosphoproteins especially their role in control of DNA synthesis and cell cycle kinetics.

BIBLIOGRAPHIC REFERENCES: Scott, R.E.: Aberrant cell cycle-cyclic AMP-dependent plasma membrane phosphorylation in transformed cells. J. Supramol. Struct., suppl. 3:202, 1979. Scott, R.E.: Modulation in cyclic AMP-dependent plasma membrane phosphorylation during the cell cycle and cell differentiation is defective in transformed cells. Fed. Proc., 38:919, 1979.

103. CYCLIC NUCLEOTIDE-INDEPENDENT PHOSPHORYLATION

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The role of cyclic nucleotide-independent phosphorylation reactions in the hormonal control of cell metabolism, proliferation and differentiation will be examined. Three cyclic nucleotide-independent protein kinases from reticulocytes, previously obtained in a highly purified form, will be examined in detail. In addition, three other cyclic nucleotide-independent protein kinases which have been identified and partially purified from reticulocytes will be prepared in a highly purified form and characterized. The two major forms of phosphoprotein phosphatase from reticulocytes will also be purified further and characterization of the physicochemical properties of the enzymes will be completed. The mode(s) of regulation of the protein kinases and phosphoprotein phosphatases will be identified. Antibody to the purified enzymes will be prepared and used to quantitate the levels of the various enzymes under conditions of hormonal stimulation. Several cell types which are insulin sensitive will be examined and the protein kinase and phosphoprotein phosphatase activities will be correlated with alterations in the phosphorylation patterns.

104. REGULATION OF THE CELL CYCLE AND DIFFERENTIATION OF NEUROBLASTOMA CELLS IN VITRO - DYNAMIC PROPERTIES OF PLASMA MEMBRANE RECEPTOR SITES

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OBJECTIVE: To reveal the molecular mechanism underlying the regulation of growth and differentiation of neuroblastoma cells, in particular the possible role of modulation in the expression of plasma membrane receptor sites.

APPROACH: The intrinsic modulations in the expression of a number of specific plasma membrane receptor sites is investigated. A possible relationship between the expression of receptor sites and their dynamic properties, measured as their lateral and rotational mobilities, is under study, with emphasis on the role of lipid-protein interactions.

105. EGF AND ITS RECEPTORS IN EMBRYONIC DIFFERENTIATION

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The role of epidermal growth factor (EGF) and its receptors (EGF-R) during embryonic development and during the differentiation and loss of tumorigenicity of teratocarcinoma cells will be studied.

Epidermal growth factor receptors have been demonstrated on the cell surface of certain differentiated teratocarcinoma cell lines. Undifferentiated embryonal carcinoma (EC) cells are malignant and totipotent, but their differentiated cell products have little or no tumorigenicity. The former cells do not have significant numbers of 125I-EGF binding sites and the hypothesis is that in the teratocarcinoma system, growth control may be governed by EGF and its receptors (EGF-R). In many respects, teratocarcinoma cells are a model of mouse development, and this suggests that the tissues of the mouse may become subject to growth control during development either by a) the appearance of EGF-R and maternal EGF or by b) the synthesis of EGF as well as EGF-R. Other markers of endoderm differentiation will be alpha-fetoprotein, transferrin, and basement membrane collagens.

It is intended to study the first appearance and the distribution of EGF-R in mouse embryos using labeled EGF. Further studies of receptor distribution in differentiating EG cells will be made. The binding and molecular characteristics of EGF-R in various cells will be compared to find out if different forms exist. Antibodies to EGF will be prepared and used to study the synthesis of EGF by mouse embryonic tissues and in teratocarcinoma cells by immunological methods. Endogenous growth factors produced by EC cells will be sought. We hope to establish embryonic cell lines using EGF and other factors to stimulate growth.

106. RECEPTOR DYNAMICS AND NORMAL/TUMOR CELL FUNCTION

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Several recent hypotheses suggest that cell recognition and growth are regulated in large part by epigenetic alterations in the structure of cell surface receptors as well as by changes in the receptor mobility and distribution. Bone marrow derived lymphocytes (B cells) provide a unique model for studying the regulation of eukaryotic cells since B cells possess membrane associated antigen-binding receptors. Ligands binding to the receptors may initiate either specific activation or specific suppression of cellular proliferation and differentiation. Receptors are present on precursors of antibody-forming cells as well as on immature antibody-forming cells. The receptors appear to be

lost through differentiation since they are not detectable on mature antibody-forming cells. I propose to study and compare the dynamics of receptors on a normal and a neoplastic antibody-forming cell pair following the interaction of the receptors with either a stimulator ligand or a suppressor ligand. The cells of the normal and neoplastic cell pair that I will use are identical in at least two respects, i.e., they each synthesize and secrete a structurally identical anti-dextran antibody, and each cell possesses receptors which bind dextran. Dextran B1355 stimulates the proliferation of normal anti-dextran antibody-forming cells while the same dextran, when oxidized, specifically suppresses the normal proliferative response. The oxidized dextran also specifically suppresses the in vivo proliferation of the neoplastic anti-dextran antibody-forming cell. In the present study, changes in the receptor (i.e., pattern of distribution and mobility, rate of shedding, and re-expression) following interaction with dextran or oxidized dextran will be monitered in the normal and neoplastic cell pair. An attempt will be made to integrate the receptor dynamics induced by dextran or oxidized dextran with alterations in cell function, i.e., protein synthesis and secretion, differentiation, DNA synthesis and mitosis. A causal relationship between the receptor dynamics and cell function will be tested by introducing dextran or oxidized dextran directly (without receptor perturbation) into the cell via a unilamellar lipid carrier vesicle.

BIBLIOGRAPHIC REFERENCES: Bankert, R.B., DesSoye, D. and Powers, L. Screening and Replica Plating of Anti-Hapten Hybridomas with a Transfer Template Hemolytic Spot Assay. Accepted for publication in J. Immunol. Methods, Feb. 11, 1980, in press. Bankert, R.B. and Mayers, G.L. Use of Hybridoma Technology to Assess the Antibody Repertoire to a Negatively-Charged Hapten. Accepted for publication in Transpl. Proc., Jan. 5, 1980, in press.

107. RECEPTOR FUNCTION IN LYMPHOCYTE DIFFERENTIATION

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Our objectives is to learn how receptors on lymphocyte membranes function in regulating cellular differentiation. We want to know the function of the antigen-binding receptor, whether ligands induce interaction with other types of membrane receptors, how the consequences of antigen binding are integrated with signals received from interacting cells, and how membrane organization governs receptor function. Our approach to the problem of receptor function combines two aspects: 1) developing chemical methods to visualize receptors, identify physical association or interaction of surface antigens with other membrane components, and isolate subpopulations of B and T cells on the basis of distinguishing surface markers; and 2) correlating the physical characterization of lymphocyte subsets with their function in a well-defined and readily manipulated in vitro anti-hapten antibody response. Our methods emphasize the use of hapten-sandwich procedures (hapten-conjugated antigens, antibodies, and cell surfaces in combination with anti-hapten antibodies) to induce the selective bridging of various receptors on the same or on different cells, to purify cells that bear particular receptors, and to deliver effectively biologically active molecules to selected cell populations.

BIBLIOGRAPHIC REFERENCES: Henry, C., B. Doe, J. Kimura, J. North and L. Wofsy. Fluorescence visualization of Ia antigens on T cells. Cell. Immunol. 53, 125 (1980). Wofsy, L. Hapten-sandwich labeling. Protein Digest 4, 18 (1980).

108. SURFACE ARCHITECTURE OF NORMAL AND CANCER CELLS

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OBJECTIVE: The main differences between cancer and normal cells (i.e. invasiveness of other tissues and failure to stop growing) are explicable in terms of an altered cell surface. The nature of the alteration is as yet unclear. In this project, the disposition of the surface components will be examined.

APPROACH: Two experimental situations are being developed. The first concerns the behavior of thymus cells in culture. The second concerns the isolation of human cell mutants that grow normally at 37 degrees, but are unable to grow at 33 degrees. In each case the aim is to establish the manner in which cells stop growing, and then to investigate the surface changes that accompany cessation of growth.

PROGRESS: Thymus cells have been separated according to size and their growth characteristics determined. The surface properties of such cells are being investigated.

109. SURFACE MEMBRANE COMPONENTS AND CONTROL OF CELL GROWTH

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OBJECTIVE: To establish the role of surface components and surface events in controlling the physiological state of untransformed cells and their ability to progress through the cell cycle as compared with transformed cells.

APPROACH: A. Macromolecular components produced by cells in the absence of serum are fractionated and tested for their ability to affect cell growth. Two fractions with an inhibitory effect are being examined in relation to the stage of the cycle at which they exert their effect. Studies are in progress to isolate the active components. B. Structural and biochemical events at the cell surface membrane are being investigated where growth inhibition is observed.

110. EXTRACELLULAR MATRIX AND CELL SURFACE IN TERATOCARCINOMA DIFFERENTIATION

Leivo, I., Vaheri, A., Virtanen, I., Wartiovaara, J., Helsingin Yliopisto, Dept. of Electron Microscopy/Pathology, *Fabianinkatu 33, SF00290 Helsinki, Finland*

OBJECTIVES: Characterize changes which occur in extracellular matrix and cell surface during teratocarcinoma differentiation, and understand the significance of these elements in differentiation.

APPROACH: Immunoelectron microscopy, fluorescence microscopy, lectin probes, histoenzymology, biochemical characterization.

PROGRESS: Projects completed: Fibronectin glycoprotein as a marker of teratocarcinoma differentiation. In preparation: Appearance of extracellular matrix material during endodermal differentiation of teratocarcinoma cultures.

111. CELL SURFACES AND GROWTH OF PANCREATIC CARCINOMA

Warren, J. R., Reddy, J. K., Northwestern University, Chicago Campus, School of Medicine, Dept. of Pathology, *303 E. Chicago Ave., Chicago, Illinois*, 60611, United States of America

The goal of this project is to characterize membrane surface properties of pancreatic acinar carcinoma cells, and relate surface properties to growth of the acinar carcinoma. A mechanical fragmentation procedure has been developed for examination of the carcinoma in vitro. The fragments demonstrate normal rates and patterns of protein and DNA synthesis when maintained in vitro for up to 5 hours. The fragments were responsive to the secretagogue carbamylcholine between 10 to the minus 8th power and 10 to the minus 3rd power M, suggesting good preservation of membrane receptors for secretagogues during tumor fragmentation. However, the carcinoma fragments demonstrated an apparent low rate of carbamylcholine-induced protein secretion as compared to normal pancreas in vitro. We suggest that the apparent low rate of protein secretion reflects functional immaturity of the acinar carcinoma. Future work will focus on further characterization of membrane receptors, protein secretion, and cell proliferation in the carcinoma.

BIBLIOGRAPHIC REFERENCES: Warren, J.R., Becich, M.J., and Reddy, J.K. Macromolecular synthesis and secretion by fragments of pancreatic acinar cell carcinoma. Federation Proceedings 39: 639 (1980).

112. BIOCHEMISTRY OF CELL DIVISION IN BACTERIA

Wu, H. C., Lai, J., Tokunaga, M., Giam, C., U.S. Dept. of Defense, Uniformed Services University of the Health Sciences, School of Medicine, Dept. of Microbiology, *4301 Jones Bridge Rd., Bethesda, Maryland*, 20014, United States of America

Control of cell division is one of the most crucial problems in cell biology which is relevant to the etiology of neoplasia in animals and human beings. Abnormal cellular morphology and

uncontrolled cell division are two of the most common manifestations of a malignant cell.

The proposed research concerns the study of control of cell division in bacteria as a model system. Bacterial mutants defective in the synthesis, assembly and degradation of the cell surface components will be isolated and characterized both biochemically and genetically. studies on the structure, function and assembly of membrane proteins will lead to a better understanding of the structural or functional alterations in cellular membrane and its interaction with the environment in normal and pathological conditions.

113. CELLULAR CONFIGURATION AND SURFACE MODULATION OF GROWTH

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This is part of a broader project. A summary of this subproject is not available.

114. CELL SURFACE PROTEINS DURING DIFFERENTIATION (FUNGI)

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This is part of a broader project. A summary of this subproject is not available.

C. HORMONES AND OTHER FACTORS IN DIFFERENTIATION AND GROWTH

1. ROLE OF HORMONES AND GROWTH FACTORS IN MAMMARY TUMOR DIFFERENTIATION AND PROLIFERATION

115. HORMONE AND RECEPTOR REGULATION OF MAMMARY CANCER

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OBJECTIVE: The role of hormones and their receptors in the growth and development of mammary carcinoma under both in vivo and in vitro conditions will be studied. In order to better understand the hormonal dependence of mammary tumors, two experimental models (DMBA and NMU) will be employed and effects on tumor growth and regulation of hormone receptor levels will be studied.

Prolactin receptors will be purified from rabbit mammary glands. This will permit the production of antisera specific to the receptor protein and allow a more accurate and reliable means of measuring prolactin receptors, with the hope of better understanding the role of prolactin in the development of human breast cancer. In addition, this approach will be important to follow the intracellular migration of the prolactin receptor.

APPROACH: Organ cultures of DMBA and NMU-induced rat mammary tumors will be established and the effect of various steroid and peptide hormones as well as cellular and metabolic inhibitors will be investigated for their role in tumor growth. In addition, the regulation of receptors for estradiol, progesterone, dexamethasone as well as of prolactin will be examined and correlated with receptor regulation in normal mammary tissue. The role of prolactin in the growth of NMU-induced mammary tumors will be examined in detail in vivo studies using the prolactin lowering drug CB-154, as well as a specific antiserum to prolactin and the prolactin receptor. These proposed studies should help clarify the regulatory mechanisms involved in tumor growth as well as possibly offering new approaches to investigate the mechanisms by which prolactin induces its actions in its target tissues. Prolactin receptors will be purified from rabbit mammary glands by two stage affinity chromatography. Conventional and monoclonal antibodies will be produced, and these will be utilized for in vivo and in vitro studies to follow the intracellular fate of prolactin receptors following internalization.

116. ROLE OF HORMONAL FACTORS IN THE CONTROL OF GROWTH AND FUNCTION OF NORMAL AND NEOPLASTIC MAMMARY EPITHELIAL CELLS

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OBJECTIVE: Role of hormonal factors in the control and function of neoplastic mammary epithelial cells.

APPROACH: Membrane receptors for prolactin are clearly important in mediating and regulating the function of prolactin in mammary cells. It is also clear that prolactin has a role in mammary tumorigenesis. However, the mechanisms by which prolactin and its receptor mediate those processes are not known. I have proposed to tackle these problems by using mammary epithelial cells maintained in tissue culture as models. 1) Initially a number of techniques to identify prolactin receptors in cultured mammary epithelial cells will be examined. The specific binding of 125I-labeled prolactin to intact cells and to membranes isolated from these cultured cells will be determined. Immunofluorescence and immunolocalization will be used to examine receptor mobility and intracellular localization. 2) The physiology of prolactin receptors in cultured cells under various growth conditions will be examined. These include variations in receptor activity throughout the cell cycle and under various growth situations. 3) The pathophysiology of prolactin receptors in relationship to mammary tumor cells will be examined. The receptor activity in normal and mammary tumor cells will be compared and correlated with their degree of hormonal dependency. The molecular mechanism(s) by which receptors mediate the growth effect in these cells will be examined using antibodies to the receptor. 4) The detailed mechanisms by which prolactin receptors can be regulated by various hormonal influences will be explored. The biosynthesis and degradation of prolactin receptors during receptor induction by hormones and in tumor cells will be examined.

These studies may shed light on the mechanism of hormonal influence on normal and malignant growth of mammary cells. The use of human epithelial cell lines in these studies may generate information which may be relevant to breast neoplasms in man.

117. EFFECT OF HORMONES ON THE GROWTH OF BENIGN AND MALIGNANT HUMAN MAMMARY TUMORS IN ORGAN CULTURE

Calaf, G., Cabezas, J., Maluenda, R., Universidad de Chile, Departamento de Ciencias Naturales, *Casilla 1240, Antofagasta, Chile*

GENERAL OBJECTIVES: a) To study the effect of hormones on cell growth in the normal and neoplastic human mammary gland in organ culture. b) To correlate this growth with several physiological and histopathological aspects of the patients.

SPECIFIC OBJECTIVES: a) To analyze the proliferative activity caused by polypeptide and steroid hormones, based on the uptake index and specific activity of normal and neoplastic human mammary tissue in culture for 5 days. b) To correlate the proliferative activity in these tissues with age, parity and histopathology in the patients.

METHODS: Cultures are being used as the experimental model since they offer responses to determinations that are not possible with in vivo methods. Using this model we will analyze the direct effect of each hormone on cell proliferation without the impediment of systemic factors. Through the use of autoradiographic studies we will be able to determine the types of cells that are affected by the hormones, which will make it possible to find foci of proliferation in mammmary tissue.

PROGRESS: Tissues obtained from 23 patients were processed into explants and each tumor was individually cultured at 37 degrees C (95 percent O2, 5 percent CO2) in Medium 199. All media contained penicillin G (50 I.U./ml), hydrocortisone (1.0 microgram/ml) and insulin (5.0 micrograms/ml). Each biopsy specimen was divided into 2 groups: Group I as a control and Group II received estrogen (E) (1.0 microgram/ml).

When E was added to the synthetic media containing explants of benign breast tumors a significant increase (p less than 0.05) in (3H)-thymidine incorporation into DNA was observed in comparison to controls.

The most important feature of this study was the demonstration that these tumors respond directly to the effect of estrogen, as indicated by increased DNA synthesis after 5 days of culture. (Translated)

118. ENDOCRINE CONTROL OF NORMAL AND NEOPLASTIC CELL PROLIFERATION - STUDIES ON ESTROGEN RECEPTOR

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OBJECTIVE: In general, we plan to continue along the research lines that we have been pursuing over the last few years, with the aim of contributing to progress in the understanding of (1) the mechanism of estrogen control of gene expression leading to proliferation of responsive cells (control of proliferation appears to be a special case of control of gene expression in eukaryotic cells) and (2) the alterations of this mechanism in cancer. We will be pursuing fundamental goals but we will be alert to practical applications, in particular greater appreciation and exploitation of the phenomenon of breast cancer responsiveness to castration and other endocrine manipulations of the host.

Our main long-term aim is to learn about the nature of the alteration(s) of growth control in neoplastic cells. However, understanding of the normal mechanism of growth control - which still escapes us - is, we believe, a prerequisite of understanding the alteration(s) of this mechanism in cancer.

We view the estrogen-target cell system as particularly suited to experimental investigations of growth control because in this case the initial controlling factor is known and appears in the form of a well-defined chemical structure, the steroid hormone.

APPROACH: We plan to apply the following basic experimental design in order to achieve the above specified aims: (1) identify the components of the cellular mechanism which is capable on one hand of recognizing estrogen, and on the other of inducing specific gene expression leading to cell growth; (2) purify and characterize these components; (3) study their mode of interaction under simplified conditions in vitro in order to understand the nature and reconstruct the mechanism by which the hormone activates gene expression; (4) identify the alterations of this mechanism in neoplastic cells by parallel study of cancer estrogen target tissues.

119. IN VITRO STUDIES OF HUMAN BREAST EPITHELIUM

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OBJECTIVES: To study parameters which control in vitro breast epithelial growth and differentiation.

APPROACH: Human breast milk contains cells which are capable of being grown in cell culture in vitro. The cells which set down in appropriate media produce colonies which we have shown to be clonal (by G-6-P.D. marker). These colonies are epithelial in origin and very closely resemble either duct cells or a combination of duct and alveolar cells. All cells contain prekeratin and are thus clearly epithelial. The colony forming cell is capable of self-renewal.

PROGRESS: Studies are currently under way to investigate the affect of a variety of hormones and other agents on differentiation as judged by the synthesis of lactalbumin, casein and lactose synthetase. The breast cultuure cells are capable of metabolizing androstenedione to testosterone and estrogens. Studies are proposed to examine the role of self-renewal and metabolism of steroids, etc. from patients with no defined risk for breast cancer in comparison to a group of high risk donors with strong family histories of breast carcinoma.

120. STEROID HORMONE REGULATION OF GROWTH IN MAMMARY TUMOR CELL LINES

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OBJECTIVE: To analyze the molecular mechanism of the regulation of growth by estrogens and glucocorticoids in human and rat breast cancer cell lines.

APPROACH: The MCF 7, ZR 75, RBA and NMU cell lines (kindly provided by Dr. M. Rich, M. Lippman and P.C. Chan respectively) are responsive to estrogens, antiestrogens and glucocorticoids. We would like to determine whether these hormones are stimulating cell proliferation directly or via growth factors secreted by these cells. In a first step, the protein and peptides induced specifically by the hormones will be characterized. In a second step, the effect of these proteins on cell growth will be tested in cells growing in a chemically defined medium. These studies might help to improve the control of the growth of breast cancer.

121. CONTROL MECHANISMS OF MAMMALIAN CELL GROWTH

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We propose to study the mechanisms which regulate the multiplication of hormone-dependent rat mammary carcinoma, in particular to identify the hormones and factors which tumor cells require for growth using in vivo and in vitro systems, and to examine how these factors and hormones regulate growth. We will use hormone-dependent (HD) and hormone-independent (HI) tumors which originated from a rat (AxC) mammary carcinoma and clonal cell lines isolated from these tumors which possess specific growth requirements in vitro. We will also use primary culture of normal rat mammary gland. The HD cell line requires for growth MGF (identified to be phosphoethanolamine) in calf serum-supplemented medium or phosphoethanolamine and a group of hormones in serum-deprived medium.

We will study the mode of action of phosphoethanolamine first by determining whether phosphoethanolamine acts like a hormone or a nutrient. The synergistic effect with other hormones will also be investigated. We will further determine the functional specificity of phosphoethanolamine. This study should show us whether phosphoethanolamine is effective only on certain mammary tumor cells or on normal mammary tissue as well, and whether it can also act on cells from other tissues and organs.

The HD tumors produce significant amounts of alpha-lactalbumin, a component of lactose synthetase. In addition, the tumors and their clonal cell lines in culture produce a large amount of fat. Once we determine whether the fat synthesized is milk specific, we intend to analyze fat and alpha-lactalbumin (and possibly casein) in both tumors (in vivo and primary culture) and cell lines under varied hormonal environments, in order to understand how growth and differentiation capabilities of the cells are regulated.

122. HORMONAL CONTROLS OF GROWTH AND DIFFERENTIATION IN CULTURED NORMAL AND NEOPLASTIC MAMMARY CELLS

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Hormonal responsiveness of several systems has been shown to vary as a function of the substratum with which cells are associated. This phenomenon is being studied in vivo and in vitro by characterizing steroid receptor levels, hormonal responsiveness and the effect of an inhibitor of extracellular matrix collagen deposition on NMU-induced rat mammary tumors. The primary NMU-tumors have deposition on NMU-induced rat mammary tumors. The primary NMU-tumors have estrogen receptors and exhibit estrogen responsiveness in vivo. Estrogen receptor levels were found to irreversibly diminish to 20% of the original levels during collagenase for tumor cell isolation. This effect was not due to an irreversible loss of collagen production by the cells since collagen synthesis resumed in culture while estrogen receptor levels remained low and since collagenase digestion at 30 degrees C rather than 37 degrees C nearly completely prevented the loss of estrogen receptor levels. The tumor cell growth was inhibited by a selective block of collagen matrix formation in vivo and in vitro. This inhibition in response to cis-hydroxyproline was shown to be specific since

transplantable NMU-tumors and MCF-7 cells did not synthesize type IV collagen and were resistant to cis-hydroxyproline.

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123. HORMONAL REGULATION OF GROWTH, DIFFERENTIATION AND TUMORIGENESIS OF HUMAN BREAST TISSUES IN VIVO

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OBJECTIVES: A. To define the hormonal requirements for growth of human breast tissues in vitro and in vivo: 1. Organ cultures (explants) of normal human breast tissues will be maintained for 2-4 days in a hormone enriched chemically defined culture media. Growth of the explants will be assessed by 3H-thymidine incorporation into DNA and mitotic figure analysis. 2. Slices of normal human breast tissues will be transplanted to and maintained (1-6 months) in hormone treated athymic 'nude' mice. Growth of the grafts will be assessed by 3H-thymidine incorporation into DNA and mitotic figure analysis. B. To define the hormonal requirements for differentiation (lactation) of human breast tissues in vitro and in vivo: 1. Organ cultures (explants) of normal human breast tissues will be maintained for 4 days in a hormone enriched chemically defined culture media. Differentiation of the explants will be assessed by histological analysis and by alpha-lactalbumin radioimmunoassay. 2. Slices of normal human breast tissues will be transplanted to and maintained (1-6 months) in hormone treated athymic 'nude' mice. Differentiation of the grafts will be assessed by histological analysis and by alpha-lactalbumin radioimmunoassay. C. To induce neoplastic transformation of normal human breast tissues in vitro-in vivo by chemical carcinogens: 1. Organ cultures (explants) of normal human breast tissues will be maintained for 2 days in a carcinogen enriched chemically defined culture media. After culture, the explants will be removed and transplanted to and maintained (1-12 months) in hormone treated athymic 'nude' mice. Neoplastic transformation of the transplanted human breast tissues will be assessed by periodic palpation and histological analysis of the grafted tissue.

124. THE ROLE OF THYROID HORMONES IN GROWTH AND DIFFERENTIATION OF MOUSE MAMMARY GLANDS

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This project is designed to understand the role of hormones (particularly prolactin and T3) in normal mammary gland development and differentiation. We also wish to determine what impact alterations in the normal thyroid hormone-prolactin balance have on mammary tumorigenesis. In addition, we wish to define any 'critical periods' where such an imbalance imprints the mammary gland and results in alteration in subsequent tumor formation. Studies include: 1) evaluation of the effect of mild hypothyroidism during the pre-pubertal period as well as throughout life on formation and/or regression of hyperplastic alveolar nodules (HAN) and ultimately on mammary tumor formation; 2) examination of the ability of hypothyroidism to prevent HAN and tumor formation in animals whose tumor potential is increased by perinatal exposure to high doses of estrogens or anti-estrogens; 3) to determine what effect perinatal estrogen treatment has on pituitary function and lactogenic receptors; 4) to characterize the nature of interaction of prolactin and human growth hormone with native as well as cryptic forms of lactogenic receptors, and 5) to partially purify the lactogenic receptor in order to produce monoclonal antibodies against it.

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125. ROLE OF ESTROGEN AND OTHER HORMONES IN BREAST CANCER

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The objective of this project is to determine the role that hormones, in particular estrogen and progesterone, play in the growth of mammary tumors. We propose to test the hypothesis that the role of estrogen in breast cancer is to act not as a mitogen, but rather to induce in the cells properties essential or related to the malignant state, including not only tumor growth but also invasion and metastasis.

We will use the human breast cancer cell line MCF-7 for these studies. MCF-7 requires estrogen in order to form tumors in nude mice but is not dependent on estrogen for growth in culture. Clones of MCF-7 differing in their properties will be compared for tumorigenicity, properties in culture associated with malignancy and the effect of hormones on both.

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126. MECHANISMS OF HORMONE DEPENDENCE OF HUMAN MALIGNANCY

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We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings. A) We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous disease, melanoma, colon carcinoma, ovarian cancer and male breast cancer. B) Assays for specific gene products (alpha-lactalbumin, casein, fatty acid, synthetase, thymidine kinase) have been developed and we are studying the effects of steroid hormones, insulin and vasopressin on the activities and synthesis of these proteins.

BIBLIOGRAPHIC REFERENCES: Koeffler, H.P., Golde, D.W. and Lippman, M.E.: Glucocorticoid sensitivity and receptors in cells of human myelogenous leukemia lines. Cancer Res. 40: 563-566, 1980. Lacroix, A. and Lippman, M.E.: Binding of retinoids to human breast cancer cell lines and their effects on cell growth. J. Clin. Invest. 65: 586-591, 1980.

127. GROWTH FACTORS AS INTERMEDIATES IN ESTROGEN-PROMOTED MAMMARY TUMOR GROWTH

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This proposal is a Faculty Research Award application to the American Cancer Society requesting five years salary support for the principal investigator. The studies described in the application are aimed at determining whether estrogens promote the growth of mammary tumors in vivo via an indirect or secondary mechanism. One possible role of estrogens in growth in vivo is to induce production and/or secretion of polypeptide growth factors by estrogen-target tissues, which then enter the general circulation and cause tumor cell growth. We have prepared models to be tested, and approaches to this problem including purification of these new 'ESTROMEDIN' activities, and characterization of their mechanisms of action in mammary tumor cells.

2. ROLE OF HORMONES IN PITUITARY, LIVER, GASTROINTESTINAL AND UROGENITAL CELL DIFFERENTIATION AND PROLIFERATION

128. ESTROGEN MEDIATED PITUITARY TUMOR CELL GROWTH

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The overall objective of this proposal is to define the role of estrogen-induced uterine and kidney derived growth factors in the proliferation of rat pituitary tumor cells in vivo and in vitro. The rat pituitary tumor cell line to be used in these studies was established by this laboratory as a subclone of the original GH3 cell population. Our studies with the GH3/C14 cell line have shown that estrogens are required for optimal growth in vivo, but are not capable of promoting continuous growth of these cells in vitro. We have condsidered several possible explanations for these observations in culture, however, our most recent approach has been to ask whether the mitogenic role of estrogens in vivo may be mediated by steroid hormone control of the production and secretion of growth factors specific for the hormone-responsive pituitary tumor cells. To evaluate the possibility of a mediated mechanism involving estrogen-induced polypeptide growth factors, we first prepared extracts of tissues removed from estrogen-treated and estrogen-deficient animals and assayed for growth activity by addition of extracts to cells in serum free medium. Our experiments demonstrate that estrogens elevate the levels of growth factors for GH3/C14 pituitary cells in extracts of both rat uterine and rat kidney tissue. These extracts promote growth of another cell type which also forms hormone-responseive tumors (MTW9/PL rat mammary tumor cells) in appropriate hosts, but do not promote growth of such non-estrogen related cells as 3T3 mouse fibroblasts. Other experiments have identified GH3/C14 cell growth factor activity in estrogen-induced uterine luminal fluid secretions, suggesting that this activity can reach an extracellular compartment and, therefore, may be capable of reaching the general circulation. To date, our experiments suggest a model of hormone-responsive pituitary tumor growth in vivo which includes the mechanism estrogens affect uterus and kidney producing specific polypeptide growth factors producing estrogen-responsive tumor cells.

129. PITUITARY HORMONES IN NORMAL AND NEOPLASTIC GROWTH

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The overall objective of this project is to elucidate the molecular mechanisms involved in the regulation of the growth and function of adrenocortical cells. It is proposed to investigate the specific biochemical events in the cell cycle which are affected by adrenal growth factors and ACTH. The molecular mechanism of induction and maintenance of the steroidogenic capacity of adrenocortical cells will be investigated. The properties of the adrenocortical cell surface as well as the cytoskeleton and the changes induced by hormones will be examined. It is also proposed to investigate the proliferation and function of normal and tumor Leydig's cells.

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130. CHARACTERIZATION OF A LIVER SPECIFIC GROWTH PROMOTOR

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The availability of a truely tissue specific growth promotor would provide a unique opportunity to investigate a wide variety of basic phenomena, including the control mechanisms involved in normal and malignant tissue growth and the role of growth promotors in the response to various toxins and tumor promotors.

I have previously reported that normal weanling and regenerating adult rat livers contain a substance(s) (HSS) that stimulates liver growth in vivo and in vitro; an increase in the rate of DNA synthesis and an actual increase in cell number are observed and this stimulation is specific for the liver. The active material has been purified approximately 1700-fold by a combination of heat, ethanol and ammonium sulfate precipitation and ion exchange and gel filtration chromatography. The active material exhibits a MW of 20-25,000 and produces its effect only after a significant lag period, during which new RNA and protein must be synthesized in order for the stimulation of DNA synthesis to occur. The objectives of the current proposal are to complete purification of HSS to homogeneity and examine its mode of action. Purification will utilize isoelectric focusing, lectin columns, cyto-affinity and gel electrophoresis techniques. HTC cells will serve as the primary screening assay, due to their marked sensitivity to HSS. However, active fractions will be tested on normal hepatocytes in culture and in vivo. A purified material will allow development of an immune assay and identification of the tissue source of HSS, its presence in various growth states (normal and malignant) and its possible production in response to toxins and tumor promotors. Important questions concerning the time course of HSS stimulation, necessity for de novo protein and RNA synthesis, cell cycle specificity and effects on protein synthesis and degradation can be approached with the present, 1700-fold enriched preparation. HSS will also be radioiodinated to determine if the specificity of its action and the variability of cellular responses to HSS are due to differences in binding to a specific receptor for HSS.

131. CYTOPLASMIC FACTORS IN CELLULAR GROWTH

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The aims of this research are to identify the substances that serve as physiological regulators of hepatocyte proliferation, and to define the mechanisms through which they operate. Previous studies from our own and other laboratories, carried out mainly in whole animals, have implicated insulin, glucagon and epidermal growth factor (EGF). We have now turned to primary hepatocyte monolayer cultures for more definitive evaluation of these three substances, and to seek additional regulatory substances which we suspect may also be required. We have established a serum-free system for short-term culture of hepatic parenchymal cells in a chemically defined medium, in which contamination with other types of cells is negligible. We are beginning to test sera from normal and partially hepatectomized rats, and will attempt to define and isolate the active principles. We are also exploring effects of a variety of nutrients, hormones, and known growth factors and their interactions and possible modes of operation.

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132. EFFECT OF VASOACTIVE INTESTINAL PEPTIDE ON CELL GROWTH - ROLE OF CAMP AND DEPENDENT PROTEIN KINASE IN TUMORAL CELL PROLIFERATION

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OBJECTIVE: 1) To investigate the role of peptide hormones (especially VIP) in cell growth and to answer the following question: Does VIP interfere with the proliferation of tumoral cells in the colon? 2) To investigate whether the unusual accumulation of glycogen in human colon tumoral cells is related to the process of cell growth.

APPROACH: HT29 cells (adenocarcinoma cells from human colon) are cultured in defined media. VIP, at 37 degrees, stimulate cAMP synthesis with subsequent activation of cAMP dependent protein kinases. We have characterized, by gel electrophoresis technique, 3 cAMP dependent protein kinases activated by VIP. VIP also activates a cAMP phosphodiesterase which is now partially purified. The role of the cAMP system (cyclase, phosphodiesterase, kinase and cAMP) in the control of cell proliferation will be investigated in great detail for two reasons: 1) VIP and IBMX (an inhibitor of phosphodiesterase) stimulate cell proliferation; 2) VIP induces glycogenolysis.

PROGRESS: The purification of the VIP receptors is in progress.

133. THE INFLUENCE OF HORMONES ON CANCERS OF THE GASTROINTESTINAL TRACT AND LUNG

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OBJECTIVES: 1) To evaluate the role of neurotransmitter substances, local hormones and systemic hormones in the control of cell proliferation and cell differentiation in tumors of the gastrointestinal tract and lung. 2) To examine the role of cyclic nucleotides as messengers for the control of proliferation and differentiation in cancer cells. 3) To determine new therapeutic regimes based on results of studies carried out under 1) and 2).

APPROACH: 1) The tumor models used are: a) chemically induced tumors of rat colon and stomach and b) xenografts of human tumors propagated in immune deprived mice. 2) Tumor biology explored by cell kinetic morphologic and biochemical techniques.

134. INTESTINAL CELLS - GROWTH REGULATION AND CARCINOGENESIS

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The overall objectives of the proposed research are: (1) to study the growth regulation of normal versus tumor intestinal cells in vitro. These studies will focus on the effects of various hormones (Hydrocortisone, thyroxin, glucagon, insulin, gastrin, etc.) and of an apparently specific growth inhibitor partially purified from intestinal villous cells on normal intestinal epithelial cells and on tumor cell lines derived from dimethylhydrazine-induced tumors of the small and large intestine. (2) To study the susceptibility to chemical carcinogenesis of normal epithelial cells derived from different portions of the rat intestine. Malignant transformation will be induced by addition of various chemicals (benzo(a)pyrene, 1, 2-dimethylbenz (a) anthracene, methylnitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine) and monitored by following many possible cellular changes (a different morphology, increased saturation density, growth in soft agar, plasminogen activator production, decreased fibronectin deposition). (3) To attempt to establish normal epithelial cell lines from human small intestine; if obtained, these cell lines will be characterized by morphologic and immunologic criteria similar to our studies of rat intestinal cells.

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135. GLYCOGEN SYNTHESIS IN HUMAN FETAL AND CANCER CELLS OF THE COLON

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OBJECTIVE: To study the kinetics of glycogen synthesis in human fetal and cancer cells of the colon in culture, its role in cell proliferation, and the effect on cell proliferation of the hormonal and biochemical agents which take part in the control mechanisms of glycogen synthesis and degradation.

APPROACH: Human fetal colon cells and cancer cells from lines HT29 and HRT18 are grown in culture and assayed for glycogen content, phosphorylases and glycogen synthetase activity in correlation to the cell count in asynchronous cultures, and to the cell cycle in synchronous cultures. Beta adrenergic receptors and receptors to various polypeptide hormones will be studied with the radiolabeled hormones. The effect of the specific effectors on the activation of adenyl cyclases, phosphorylases, and glycogen synthetase, as well as on the modifications of cAMP and glycogen content in the cells will be studied. The different modifications will be correlated to cell proliferation.

PROGRESS: Results showing the presence of high stores of glycogen in human fetal colon cells, and in both carcinomas of the colon and permanent cancer cell lines, have been published as a preliminary communication (C.R.Acad.Sci., 1977, 284, 105) and support the present project.

136. HORMONAL REGULATION OF KIDNEY EPITHELIAL CELL GROWTH

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The proposed research will evaluate the mechanisms by which hormones regulate the growth of kidney epithelial cells, and the alterations which occur upon malignant transformation. The MDCK cell line is particuarly advantageous as compared with other renal cell lines, for studies concerning hormonal regulation of growth as Medium K-1, a hormone supplemented, serum-free medium, has been developed which permits MDCK cells to grow in the absence of fetal calf serum. Following the methodologies developed in the laboratory of Dr. Gordon Sato, five supplements were identified (insulin, transferrin, T3, hydrocortisone and PGE1) which permitted MDCK cells to grow over a long term, and at an equivalent rate in serum-free and serum-supplemented medium. Medium K-1 has also been used to grow primary cultures of baby mouse kidney epithelial cells. Medium K-1 will be used for the following studies: (1) The possibility that PGE1 stimulates growth by increasing intracellular cAMP in MDCK cells will be examined. Available PGE1 independent variants of MDCK cells (variants which lack PGE1 requirement for long term growth in serum-free medium) will be utilized. 2) The possibility that malignant transformation is accompanied by changes in requirements of hormones for growth will be evaluated, utilizing available tumorigenic transformants of MDCK cells. (3) Growth regulation by hormones will also be examined in primary cultures of kidney epithelial cells. Medium K-1 was utilized to select epithelial cells from primary kidney cultures, whose growth responses to hormones resemble those of MDCK cells. In order to selectively grow different types of epithelial cells in primary kidney cultures, additional hormone-supplemented, serum-free media will be developed for kidney epithelial cell line which do not grow in Medium K-1.

137. BIOCHEMICAL CONTROL OF GROWTH IN ANDROGEN-RESPONSIVE ORGANS AND NEOPLASMS

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OBJECTIVES: To determine the biochemical factors that confer hormonal sensitivity on a tissue and to develop procedures for the assessment and regulation of hormonal responsiveness and differentiation in prostatic cancers.

APPROACH AND PROGRESS: The three approaches used in this research are as follows: 1) Studies of acceptor sites for androgen-receptors: The nuclear acceptor sites for androgen-receptors are being studied in rat prostate using nuclease fractionation; techniques for covalently cross-linking receptor complexes to chromatin; and a reconstituted system involving exogenous nuclear receptor complexes. Progress: Approximately 60% of the receptors can be chemically cross-linked to chromatin in situ. Partially purified nuclear receptor complexes bind to receptor-depleted chromatin but not to isolated prostatic DNA. 2) Studies of plasminogen activator: The enzyme, plasminogen activator, is being assayed in prostatic tumors of Nb rats, in human prostates, and in breast tumors to determine whether the enzyme is a suitable marker of neoplastic growth. Progress: The concentration of plasminogen activator is 2- to 3-fold higher in

human prostatic tumors than in normal or hyperplastic prostates. Breast tumors, which are positive for estrogen-receptors, have higher levels of this enzyme than receptor-negative tumors. 3) Studies of the regulation of differentiation by androgens: The relationship between the stimulation of secretory acid phosphatase, a marker of differentiation in rat prostate, and the nuclear concentration of both unbound and receptor-bound androgens is being studied. Progress: In the regenerating prostate, maximal induction of the enzyme is observed when the nuclear concentration of receptors is approximately 25% of normal.

138. HORMONAL REGULATION OF GENE EXPRESSION IN PROSTATIC CANCER

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Emphasis has been placed on the regulation of prostatic tumor growth by the sex steroids and, recently, by prolactin. However, little progress has been made towards understanding at a molecular level the mechanisms by which steroid and peptide hormones regulate prostatic cancer. The purpose of these studies is to examine hormonal regulation of gene expression in prostatic cancer. This research will focus as follows: 1) Analysis of poly A(positive)RNA populations - this will determine the number of classes of poly A(positive)RNA and changes that may occur due to hormonal regulation of prostatic tumor growth. 2) Prostatic binding protein (PBP) mRNA and dihydrofolate reductase (DHFR) mRNA will be studied as potential markers. PBP and mRNA has been previously used as a marker of differentiated function in the normal rat ventral prostate and DHFR mRNA synthesis has been closely correlated in time with DNA synthesis. 3) Methylation of DNA will be examined to determine differences between normal and tumor tissue. Recent data has demonstrated that DNA methylation may regulate gene expression and cellular differentiation. 4) Identification of new marker proteins for prostatic cancer. Significant advances in our understanding of hormonal regulation of gene expression for highly abundant mRNAs which represent secreted proteins and the moderately abundant mRNAs which represent enzymes and proteins that are important in daily cellular function will result from these studies. In addition, this research may help establish new prostatic marker proteins which can be utilized as markers for early prostatic cancer.

139. CONTROL OF PROSTATE CELL GROWTH AND CELL DIVISION

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Intra- and intercellular events related to the control of prostate cell growth and cell division exerted by androgens are being studied at 2 levels of complexity: (a) Evidence for interactions between rat ventral prostate epithelial and connective tissue cells is examined by cultivating these separated cell types obtained from normal or hormonally manipulated rats, either alone or in combination. (b) Effects of androgens on the composition and function of DNA replication complexes isolated by a procedure originally used to separate prostate 'euchromatin' are determined.

Because of ready access to human breast cancer samples, and responsibility for estrogen and progesterone receptor measurements on this material, studies with these receptors, relevant to prostate steroid hormone receptors, are being performed. Lastly, in view of the work on protein synthesis intrinsic to cell nuclei that has already been done and the availability of a Chinese hamster ovary cell line (tsH1) with a temperature-sensitive defect in cytoplasmic non-mitochondrial protein synthesis, this cell line is being used to examine protein synthesis intrinsic to cell nuclei.

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140. PROSTATIC DIFFERENTIATION AND SEX HORMONE METABOLISM

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The overall objective of our current and proposed studies is to understand how androgens and estrogens act either antagonistically or in concert to regulate mechanisms responsible for prostate growth and differentiation. We propose to study the differential response of regressed prostatic epithelium from castrated and hypophysectomized dogs to several natural androgens and an estrogen, administered separately and in combination. In this investigation we will specify those morphological features of the responding epithelial cells and patterns of sex-hormone uptake, retention and metabolism by the affected gland that will distinguish androgen- from estrogen-controlled differentiation and antagonistic from synergistic actions on regulation of coordinated growth. We propose to investigate cell-surface alterations in non-junctional plasma membranes as well as intercellular junctions in hormonally modified prostatic epithelium. We shall apply the experience gained with the canine model to a study of human prostate carcinoma in order to explore the differential sex-hormone responsiveness of the neoplastic cells.

To accomplish these objectives we shall utilize the following investigative techniques: 1) Electron microscopy: standard thin-sectioning, special procedures for preserving and enhancing contrast of cell coats, freeze-cleave replication for detailed examination of internal aspects of cell membranes. Electron micrographs will be analyzed using qualitative and morphometric criteria. 2) Organ culture: for studying the uptake and disposition of labeled sex steroids in atrophied and hormone-stimulated regressed prostate. 3) Thaw-mount autoradiography: for visualizing sex-radiosteroid location in the various cell components of explants of atrophied and hormonally modified prostate, with parallel autoradiographic 3H-thymidine labeling.

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141. PROLIFERATION OF HUMAN ENDOMETRIAL STROMAL CELLS IN CULTURE - EFFECTS OF HORMONES

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Preliminary studies indicate that stromal cultures from endometria at different stages of the menstrual cycle show distinct proliferative responses to progesterone and estradiol.

Growth of cells derived from secretory phase endometria is inhibited by estradiol and progesterone whilst those from proliferative endometria show a variable response ranging from no effect to an 80 percent increase in cell number. Two strains from patients with benign cystic hyperplasia exhibited no change in growth response to either steroid.

The estradiol-stimulated growth of proliferative phase cells is highly dependent on serum, optimum effects being obtained in the presence of 2-5 percent serum. Dexamethasone exhibits a strong stimulatory effect at all serum concentrations and shows synergism with estradiol. Furthermore, insulin enhances stimulation by both hormones. The way in which these hormones interact to affect cell proliferation in this system is under current study.

142. HORMONAL REGULATION OF CULTURED ENDOMETRIAL CELLS

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A technique to culture epithelial rabbit endometrial cells in chemically defined medium has been developed. Estrogens were found to promote cell division, while progesterone had an opposite effect. These cultured cells were found to be either noncycling (G0) or cycling. Both cell subpopulations were isolated and it was found that the G0 cells were the target of estrogens.

Interaction between progesterone and the cycling cells was necessary to obtain an antagonistic effect on the estrogen action on the G0 cells, apparently due to the production of a diffusible factor. A similar factor, probably a protein, appears to be produced by high density cultures, which do not respond to estrogen stimulation.

Estradiol and progesterone decrease the number of estrogen receptors in cultures, apparently by different mechanisms of action. We will continue to study our experimental system by analyzing the mechanism of action of ovarian sex hormones, epidermal growth factor and prostaglandin F-2-alpha in regulating proliferation, cell cycle, blastokinin synthesis, and hormone receptors. The endpoint of our research is to improve our comprehension of the mechanism by which sex hormones regulate proliferation and differentiation of endometrium. It is expected that the study of our hormone-responsive system will help to advance the understanding of the etiology and pathogenesis of endometrial carcinoma in particular and hormone-related cancer in general.

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143. ENDOCRINE CONTROL OF NORMAL AND NEOPLASTIC CELL PROLIFERATION - STUDIES ON ESTROGEN RECEPTOR

Bresciani, F., Sica, V., Universita degli Studi di Napoli, Istituto di Patologia Generale Cattedra, *S. Andrea delle Dame 2, 80138 Naples, Italy*

The summary for this project is under entry no. 118.

3. PLATELET AND CELL-DERIVED GROWTH FACTORS IN DIFFERENTIATION AND PROLIFERATION

144. HUMAN GROWTH FACTORS

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The object of the present project is to describe the role of growth factors in normal or abnormal growth control.

Growth factors, stimulating the multiplication of human cultured glial cells are purified from human platelets and from the culture medium of a line of human osteosarcoma cells. The chemical and biological properties of purified factors are examined. This includes characterization of the interaction between platelet or osteosarcoma-derived factor and the responding cells.

145. REGULATION OF MAMMALIAN CELL CYCLE

Pledger, W. J., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Pharmacology, *Manning Dr., Chapel Hill, North Carolina,* 27514, United States of America

The current concepts of cell ragulation has led to a hypothesis that is difficult to biochemically and biologically analyze. I have developed a culture system which allows experimental study of growth cycle regulation, and I have shown the serious limitations of the previously used cell systems for the study of growth control.

Serum control of cellular growth has been shown to be regulated in two discrete phases: competence and progression. I have proposed the study of the processes involved in competence and progression. The understanding of these processes will provide valuable knowledge concerning cell regulation.

Competence is induced by a platelet derived mitogen. The biochemical processes by which competence is induced are not known. The experiments proposed in this grant are designed to elucidate the processes by which competence is formed. Since SV40 abrogate the cellular growth requirement for the platelet mitogen, it is important to determine if transformation induces the normal biochemical process which renders the cell competent.

This proposed work will allow the study, cellular and biochemical, of the process of commitment (that point at which the cell is committed to DNA synthesis). The analysis of this process will greatly enhance our knowledge of cell cycle control.

I have proposed work to show how viral transformation alters normal cell regulation. These findings can lead to a model that describes the mechanism by which normal cell regulation is lost.

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146. CHARACTERIZATION OF SERUM FACTORS INDUCING MITOSIS

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Normal animal fibroblasts cultured in vitro require the presence of blood serum factors for growth. We have purified to homogeneity a growth factor polypeptide from human serum that stimulates DNA synthesis and cell proliferation of mouse and human fibroblasts (PNAS 72:2603, 1975). Biological assays with cell culture and a specific radioimmunoassay for the human serum derived growth factor (PNAS 74:1973,1978) have demonstrated that the human serum growth factor polypeptide is largely derived from platelets. This platelet derived growth factor (PDGF), stored in platelet alpha-granules, is released into serum during clotting. PDGF has been purified to homogeneity by a process which includes heat (100 degrees) treatment of washed platelets, followed by ion-exchange chromatography, gel filtration in 1 M acetic acid, isoelectric focusing and preparative SDS polyacrylamide gel electrophoresis. PDGF has a PI of about 9.8 and a molecular weight of about 13,000 on reduced SDS-PAGE gels. These properties of PDGF are similar to those of the human serum growth factor previously isolated in our laboratory (PNAS 72:2603, 1975). The specific activity of the purified PDGF is 20 million times greater than that found in unfractionated human serum. Purified PDGF stimulated replicative DNA synthesis and cell proliferation at 10 to the minus 10th power M; about 10 to the sixth power molecules per cell stimulate a round of DNA synthesis. In cell culture, PDGF induces the initial event in the replicative response 'competence'. Platelet-poor plasma (PPP) induces a later event, 'progression,' which allows competent cells to pass through G0/G1 and enter the S phase. These two sets of factors, in platelets and PPP, act synergistically to stimulate growth of mammalian cells in culture.

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147. REGULATION OF THE MAMMALIAN CELL CYCLE BY PLATELET-DERIVED HORMONES

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It has recently been shown that circulating blood platelets sequester a cationic polypeptide hormone which is released during clot formation. This 'platelet hormone' is mitogenic for normal fibroblasts in culture and may be a principal component of serum required for growth of fibroblasts in culture.

My research will be directed toward studying the involvement of platelet hormone in neoplastic transformation and in the pleiotypic response of cells to growth regulatory stimuli, and toward exploring the mechanism whereby platelet hormone, together with plasma, induces normal cells to become competent for cell division.

148. MECHANISMS OF CONTROL OF MAMMALIAN CELL MULTIPLICATION

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Efforts will be made to identify the molecules in calf serum that stimulate resting cultures of chick embryo fibroblasts to pass through the G1 period into the S phase.

Work will continue to elucidate the mechanism by which insulin preferentially stimulates the synthesis of ribosomal proteins in the resting chick embryo cells.

Serum increases the incorporation of 3H-leucine by the chick embryo cells one polypeptide out of almost 1000 labeled molecules that can be separated on two-dimensional O'Farrell gels. The mechanisms of increased labeling and the role of the polypeptide during the G1 period will be explored.

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149. ROLE OF SERUM AND CATIONS IN MALIGNANT CELL GROWTH

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Several observations indicate that the different effects of serum and serum components on normal versus transformed cell growth are related to divalent cation metabolism. The growth of normal cells in culture can be modified by the availability of both serum and divalent cations. There is evidence that divalent cations and serum act synergistically in the control of cell growth and that this control is lost upon transformation to the malignant state. Serum or calcium deprivation inhibits proliferation of a number of normal cells in contrast to the absence of such an effect on transformed counterparts. However, elevation of medium Ca levels in serum deprived normal cells can antagonize the growth inhibitory effect of low serum. The loss of density dependent growth inhibition in 3T3 cells is characterized by a simultaneous reduction in the extracellular Ca and serum requirement. Magnesium and serum act synergistically in inducing chicken fibroblasts to traverse G1 phase and initiate DNA synthesis. Serum platelet factors are required to commit several normal cell types - but not transformed cells - to DNA synthesis. Ca can substitute in part for these factors. We propose to establish the extracellular Ca, Mg and serum requirements for growth of human diploid fibroblasts as compared to an SV40 transformed counterpart with specific reference to their ability to traverse G1 phase of the cell cycle, the phase in which growth control is exerted in the normal cell. We have achieved synchronization of both normal and transformed W138 cells using centrifugal elutriation. This will allow direct comparisons to be made between these cells using an identical synchronization procedure. We propose to evaluate what effects the manipulation of serum, Ca and Mg have on divalent cation transport and content during G1 phase progression in the normal cell and how these parameters are related to loss of growth control in the transformed cell. We propose to determine any specific changes in divalent cation management associated with the ability of platelet factor to induce competence for DNA synthesis in normal cells and to establish how such changes might relate to the capability of transformed cells to remain continually competent in the absence of platelet factor.

150. EFFECTS OF MITOGENS ON NORMAL AND NEOPLASTIC CELLS

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The objective of this project is to identify and characterize molecules that regulate cell proliferation in normal and neoplastic cells and to understand the mechanism of action of these molecules. This proposal is based on the hypothesis that growth factors regulate cell proliferation and that one way in which neoplastic transformation allows cells to become cancerous is to alter cellular growth factor requirements. The response of normal and malignant cells in vitro to two mitogens, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) will be evaluated. The growth stimulatory effects of these molecules on normal and malignant human epithelial cells, SV40-transformed rodent cells and revertants of these transformed cells, will be investigated. Mutants temperature-sensitive in their response to mitogens will also be isolated and characterized. These latter experiments should help to define the cellular processes that occur in the process of growth stimulation by mitogens. The following specific projects will be undertaken: 1. Evaluate the response of normal and malignant human epithelial cells to platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). 2. Determine if transformation by the oncogenic virus SV40 alters the cellular requirement for platelet-derived growth factor. This will be done by investigating the ability of temperature-sensitive mutants and deletion mutants of SV40 to induce DNA synthesis in cultures of rodent cells maintained in the absence of platelet-derived growth factor. 3. Determine if revertants of SV40-transformed cells have increased requirements for PDGF and EGF. 4. Measure the levels of various SV40-specific antigens within these revertant cells. This will be done by immunoprecipitation with specific antisera. 5. Isolate mutants of 3T3 cells temperature sensitive in their response to PDGF and EGF.

151. EPIDERMAL GROWTH FACTOR IN HUMAN SKIN AND DISEASE

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Human epidermal growth factor (h-EGF) will be isolated from adult urine and its role in the growth and differentiation of human skin investigated. This study will utilize fetal and adult skin to establish primary cell cultures of epidermal keratinocytes, cell lines of dermal fibroblasts, and organ cultures of whole skin. These cells in culture provide physiologically and clinically relevant systems for research in that they express many of the differentiated functions that are observed in vivo. Utilizing these in vitro models, investigations will be undertaken to identify the target cell (epidermal and/or fibroblast) for h-EGF, the nature and mechanism of hormone-cell interactions and the subsequent biological response(s) of these two cell types to h-EGF. Concurrently, the physicochemical properties and biochemical effects of the hormone will be determined in keratinocytes and fibroblasts obtained from patients suffering from such non-melanoma epithelial skin cancer as basal and squamous cell carcinoma; in a benign hyperplastic epidermal disease, psoriasis; and in connective tissue disorders such as progressive systemic sclerosis (scleroderma) and lichen myxedematosus. Experimental results from abnormal cells will then be compared with data obtained from cells of normal donors who have been age, sex, site and race matched. In addition, a sensitive radioreceptor assay has been developed to quantitate levels of h-EGF in blood. In order to determine if EGF metabolism is altered in pathological states, blood levels of h-EGF will be compared with the levels in patients presenting with a variety of cutaneous disorders.

152. GROWTH FACTORS - CONTROL OF ANIMAL CELL PROLIFERATION

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We propose to continue our work in four areas related to the role of growth factors, particularly epidermal growth factor (EGF), in the regulation of cell multiplication: the physical properties of growth factor-cell interaction, the biological response of normal and transformed cells, the specific changes in these physical and biological properties in neoplasia and aging, and the genetic control of growth factor membrane receptors. Availability of complex defined medium will allow us to examine the potency of specific putative growth factors and to evaluate potential synergism of specific combinations of these factors in stimulating cell growth. Human diploid fibroblasts will be passaged in defined medium with various combinations of growth factors and the life span compared to parallel cultures grown with serum supplement. EGF binding studies on aging cells will attempt to characterize the turnover of EGF receptors as a function of normal and precocious aging. Iodination and fractionation of membrane associated proteins, glycoproteins

and lipoproteins will set the stage for the development of techniques to isolate and compare EGF receptors from early and late passage human strains. Identical techniques will be used to characterize membrane components of normal rat kidney cells and transformed derivatives which have lost their responsiveness to EGF mitogenic effect and show little or no EGF binding. In this system, transformed cells produce factor(s) which enhance EGF binding to normal cells and stimulate initiation of DNA synthesis. Isolation and characterization of these cell products may be possible through the use of serum-free complex defined medium. Genetic regulation of EGF receptors will be studied by hybridizing cells with and without receptors to determine dominance or recessiveness of receptor expression; these studies may provide clues to the linkage of EGF receptor structural genes to specific chromosomes. Characterization of surface membrane proteins and growth factor receptors during aging and transformation may provide new knowledge of the events limiting cell division and lead us closer to methods of preventing and/or controlling malignant growth.

153. STUDIES ON GROWTH CONTROL EXERTED BY THE PROTEIN NERVE GROWTH FACTOR ON PHEOCHROMOCYTOMA CELLS (CLONE PC12)

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OBJECTIVE: Understanding the molecular mechanism(s) by which the protein nerve growth factor (NGF) blocks division and induces differentiation in a neuronal population of rat pheochromocytoma (clone PC12) cells.

APPROACH: Characterization of the interaction between NGF and PC12 cells (receptor); internalization and intracellular fate of NGF; identification of cytoplasmic structural components where NGF is located (nucleus, contractile proteins, etc.); molecular changes occurring on the cytoplasmic membrane during NGF-induced arrest of mitosis and neurite growth.

PROGRESS: There are specific receptors for NGF in PC12 cells having Kd of 2 to 10 to the minus 10th power M; after binding NGF is internalized and concentrates around the nucleus; internalization of NGF is influenced by cell density; with confluent cells internalization is blocked; half-life of NGF in PC12 cells is 18-20 hours; NGF stimulates synthesis of new receptors by 6-8 fold within 3 days.

154. NGF AND DIFFERENTIATION OF NEURONAL TUMORS

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The regulatory mechanism for control of growth rate and differentiation of normal and neoplastic neuronal cells is poorly understood. Nerve growth factor (NGF) is a polypeptide important to the normal growth and development of the sympathetic nervous system of vertebrates. NGF can decrease the growth rate, induce neurite outgrowth, and enhance activity of enzymes regulating transmitter synthesis in rat pheochromocytoma (PC12) cells and in certain human neuroblastoma cell lines. The objective is to define the sequence of molecular events culminating in the differentiated state by studying binding of NGF, by identifying differentiation-specific macromolecular components (MC), by studying the pattern of MC in wildtype and NGF-resistant variants in the absence and presence of NGF, other agonists, and antagonists of neurite outgrowth. Attempts will be made to determine whether regulation of MC is at the transcriptional, translational or post-translational level. Binding of NGF to PC12 cells will be studied in culture and in vivo and will be compared with the properties of binding to rat adrenal chromaffin cells. Binding and response to NGF of cultured human neuroblastoma lines will be compared with that to freshly cultured tumors. There is weak circumstantial evidence suggesting involvement of NGF in neuronal tumors and this proposal is directed towards study and resolution of this possibility, as well as towards furthering the basic understanding of neuronal cell differentiation.

155. MECHANISM OF NERVE GROWTH FACTOR INDUCED DIFFERENTIATION OF A PHEOCHROMOCYTOMA

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A clonal tumor cell line of pheochromocytoma, PC12, responds to nerve growth factor (NGF) in culture by ceasing cell division and extending neuron-like processes. We are investigating the mechanism by which NGF accomplishes this. In particular, we are characterizing the specific plasma membrane and nuclear receptors of the cell in terms of their thermodynamic and kinetic properties. The degree of occupancy of receptors is then correlated with the differentiation observed over a range of NGF concentrations. The internalization of NGF is being investigated using an NGF-ferritin conjugate and by following the metabolism of 125I-NGF during the induction period. In parallel, we are defining the increases in cellular RNA and protein and the cessation of DNA synthesis. The study is concentrating upon nuclear events since this will allow us to examine the ability of NGF to induce these changes in nuclei incubated in in vitro systems.

By correlating the temporal properties of these events, we wish to test the hypothesis that the binding of NGF to the plasma membrane receptor is followed by internalization of the NGF-NGF receptor complex. This complex is then processed and/or degraded by lysosomes while a portion of the NGF passes to the nucleus, interacts with the nuclear receptor and in so doing, initiates one or more nuclear events which are an integral and essential part of the subsequent morphogenesis.

156. PERSISTING ACTION OF FIBROBLAST-DERIVED GROWTH FACTOR

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Fibroblast-derived growth factor (FDGF, Bourne and Rozengurt, Proceedings of the National Academy of Sciences, USA, 1976, 73, 4555) is a potent stimulator of DNA synthesis in quiescent cultures of a variety of cell types. The peptide acts synergistically with insulin, EGF, neurohypophyseal hormones, TPA, retinoids and microtubule-disruping agents. FDGF has proved to be a useful tool in studies concerning the early molecular events preceeding the initiation of DNA synthesis. The peptide is a potent stimulator of uridine and deoxyglucose uptake, glycolysis and ion movements. One of the most striking properties of FDGF is the persistence of its action after a transient exposure to quiescent monolayers of 3T3 cells. FDGF added to quiescent cultures of 3T3 cells and kept in the nutrient medium produces a substantial stimulation of DNA synthesis. When the cultures are thoroughly washed after 60 minutes exposure to the factor, the mitogenic stimulation is drastically reduced. However, when the cells that were exposed to FDGF for 60 minutes are incubated in the presence of insulin (which by itself has no effect on DNA synthesis in 3T3 cells) there is a marked stimulation of thymidine incorporation. The 'persistence' of FDGF action has been verified in many experiments and found with different preparations of the peptide. Studies on this system should increase our understanding of the crucial early events that might be instrumental in the initiation of the cell cycle.

157. STRUCTURE AND FUNCTION OF FIBROBLAST GROWTH FACTOR

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Fibroblast growth factor, isolated from both brain and pituitary, is one of the many hormone-like substances that stimulates cell growth and division. It is presumed that these substances exert similar effects in vivo and, thus, act as regulators of cell growth for both the developing and mature animal. Studies in both forms of FGF have confirmed the reports that these are unique substances but have established that available preparations are grossly impure. In the case of brain, a major contamination, i.e., fragments of myelin basic protein, was incorrectly assigned the activity. This was disproven by three crite-

ria. Immunoaffinity chromatography and high performance liquid chromatography will be used to achieve purified preparations of both FGF and molecular characterization will be pursued, particularly for the pituitary factor. Studies to identify and isolate ovine pituitary FGF will be initiated. Both bovine and ovine FGF will be used to prepare tracers for radioimmunoassays and receptor binding experiments.

158. EFFECT OF MALIGNANCY ON CELL GROWTH REQUIREMENTS

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We will continue a detailed analysis of the growth requirements of various normal human and mouse cells and their transformed counterparts. During the coming year, particular emphasis will be given to the following: 1) Characterization of a small peptide growth factor that is produced by several types of transformed cells and stimulates their growth at clonal density; 2) identification of factors from serum protein that are needed for clonal growth of normal and transformed human fibroblasts in the best currently available media; 3) detailed analysis of the growth requirements of human epidermal keratinocytes, which we can now culture without a feeder layer or conditioned medium. The long-term goals of this study are: 1) To understand fully the growth requirements of various types of normal cells from humans and from animals used in cancer research, 2) to understand the changes in growth requirements and growth regulation that occur in malignancy, and 3) to identify differences between normal and malignant cells of potential value in future clinical treatment of malignancy.

159. FACTORS REQUIRED FOR MAMMALIAN CELL DIVISION

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The long-range objective is to understand enough about the factors that control mammalian cell growth to be able to apply this knowledge to the control of malignant growth. As a result of recent work, a broad picture is now available of the control of growth of mammalian cells. Application of this knowledge requires additional information and experimentation. Detailed information is needed on the specific factors that control the growth of the epithelial cells that are most important in the origin of tumors, in order to design rational procedures to restrict tumor growth. It is important to know whether these epithelial cells produce inhibitors of their own growth, such as the growth inhibitors that are produced by kidney epithelial cells. Isolation of the kidney determines whether the inhibitors can be used to restrict the growth of kidney tumors. These questions will be studied using methods of cell culture to investigate growth controls, using ultrafiltration, chromatography and other biochemical procedures in the isolation of the inhibitors, and using cell free systems, cell culture and work in intact animals to investigate the properties of the inhibitors.

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160. CARTILAGE- AND CHONDROSARCOMA-DERIVED GROWTH FACTORS

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Cartilage and chondrosarcoma contain growth factors capable of stimulating DNA synthesis and cell division in cultured cells. The cartilage-derived growth factor (CDGF) has been purified to homogeneity and is a polypeptide of MW 16,300, plus or minus 200, with an isoelectric point between 9 and 10. CDGF is synthesized by chondrocytes and is apparently a chromosomal protein. When chondrocytes are subfractionated into cytoplasts and karyoplasts, all of the growth factor activity is found in the karyoplasts. Furthermore, all of the growth factor activity in the karyoplast is found to be associated with chromatin. CDGF stimulates the proliferation in vitro of several cell types that are involved in bone formation. These cells include chondrocytes, osteoblasts and capillary endothelial cells. CDGF also stimulates the migration of capillary endothelial cells in vitro. Our goals are to elucidate the mechanism of action of CDGF, to find out which cells are targets for CDGF in vivo and to compare the growth factor activity of normal cartilage and chondrosarcoma. The direct objectives of the proposed research are: 1) to continue purification and characterization of CDGF, 2) to purify the chondrosarcoma-derived growth factor, 3) to investigate the mechanism of action of CDGF, 4) to investigate the role of CDGF as a stimulator of capillary endothelial cell proliferation and migration in vitro and of capillary proliferation and migration in vivo, 5) to raise antibodies against CDGF, and 6) to use CDGF to enhance the growth rate of cells in culture, particularly primary amniotic fluid cells obtained in amniocentesis.

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161. GROWTH CONTROL IN NORMAL AND NEOPLASTIC CELLS

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The multiplication of animal cells in vitro requires the presence of macromolecular growth factors in the culture medium. Serum, the usual source of these factors for cell growth, is extremely complex and the identification and characterization of serum's growth promoting components has been very difficult. In these proposed studies, a purified growth factor, called Multiplication-stimulating Activity (MSA) will be used to investigate various parameters of cell growth regulation. MSA is produced by the BRL-3A line of rat liver cells which grow in the complete absence of serum. Conditioned medium from these cells is used as the source for the purification of MSA, which is a protein of about 9,000 M.W. MSA will stimulate DNA synthesis and growth of a variety of cell types; has weak insulin-like activity and appears to be a representative of the somatomedin family of growth regulatory hormones. Various parameters of growth regulation will be examined using MSA, including biological responses as well as labeled MSA binding and utilization. IN addition, we have been successful in purifying a somatomedin, or MSA carrier protein from conditioned medium using affinity chromatography procedures with immobilized MSA linked to Sepharose 4B. We plan to characterize this protein and compare it to somatomedin carrier complexes isolated form serum and examine its role in modulating the biological activity of MSA in vitro. Using similar affinity chromatographic procedures, it will also be possible to isolate the MSA receptor from cell surfaces and characterize its properties.

162. CONTROL OF NORMAL AND ABNORMAL CELL DEVELOPMENT

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This research program has been constructed with a primary aim of investigating the controlling events concerned with normal and abnormal cell development processes. This program will bring together studies utilizing both immunologic and non-immunologic systems for approaching questions pertaining to differentiation, activation and expression of biological function of mammalian cells. The diversity of scientific disciplines among the staff scientists involved will be integrated in the context of the theme of the program. Moreover, although each individual research program constitutes an entity within itself, there are ample, as well as necessary, collaborative interactions between related programs. Studies to be undertaken include the role of certain soluble mediators in controlling various aspects of cell differentiation and function and physicochemical analysis of such mediators (Research Program I), analysis of the diversity

of sarcoma gene products and the relationship of this diversity to normal cell development and malignant transformation (Research Program II), delineation of the mechanisms of diversity of immunologic receptors on both classes of lymphocytes (Research Programs III and IV), analysis of host-tumor interactions and development of new methods for favoring host rejection of tumor growth (Research Program V) and structural analysis of murine retroviral gene products and the relationship of recombination events to leukemogenesis (Research Program VI). Core facilities to serve all of the research programs include a hybridoma facility and fluorescence-activated cell sorter facility. The collective information obtained in this program should broaden our fundamental understanding of differentiation mechanisms and of how disturbances in such mechanisms may result in abnormal cell development and function.

163. TISSUE FACTOR AND ITS RELATIONSHIP TO MALIGNANCY (HUMAN, MAMMALS)

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This is part of a broader project. A summary of this subproject is not available.

164. TRANSFORMATION AND RECOVERY OF CROWN GALL TUMOR CELLS

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We propose to continue our studies on the chemical structure and mode of action of two new types of growth-regulating substances which are synthesized persistently by plant tumor cells and which are importantly involved in the establishment and maintenance of the tumorous state in cells of higher plant species. One of these is a potent cell division-promoting factor which has been assigned the trivial name cytokinesin I while the other is a cAMP(3'5')-like compound which appears to be the pyrophosphate derivative of authentic cAMP(3'5'). We also propose to continue our studies on the regulatory mechanisms involved in the persistent but reversible suppression of the tumorous state in plants. Our studies have suggested that positive feedback control mechanisms are involved in determining whether the normal phenotype or the tumor phenotype is expressed in transformed cells. We propose to study, at the biochemical level, how these positive feedback loops are established and maintained in cells that exhibit the tumor phenotype and how those loops are interrupted when the cells show the normal phenotype.

4. OTHER STUDIES OF HORMONAL AND BODY-SITE CONTROL OF DIFFERENTIATION AND PROLIFERATION

165. USE OF NB RAT LYMPHOMAS AND LYMPHOMA CELL CULTURES FOR STUDYING HORMONAL DEPENDENCY OF TUMORS AND FOR THE BIOASSAY OF LACTOGENIC HORMONES

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The objectives are (1) to develop a bioassay for human growth hormone (hGH), human prolactin (hPRL) and other lactogens, which is suitable for use in, for instance, clinical diagnosis and investigations, (2) to develop an experimental model for studying the mechanisms underlying the progression of tumors from hormonal dependency to autonomy, (3) to define biochemical processes associated with the mitogenic stimulation of lymphoma cells by lactogens.

PRL of various species has been found to be a powerful mitogen for cells cultured from a transplantable Nb rat lymphoma (Gout, Beer and Noble, Cancer Res. 40, 2433 (1980)). The cultures can detect hormonal concentrations well within the physiological range. Recent studies have further shown that the response of the cells is specific for lactogens, including hGH and hPRL; the stimulatory effects of these hormones in a serum sample can be individually blocked by antibodies to the hormones (Tanaka, Shiu, Gout, Beer, Noble and Friesen. J. Clin. Endocrin. Metab.: In Press, Nov. 1980). It is planned to define the optimal conditions for the use of the cultures in the specific bioassay of the hormones in sera and tissues, giving attention to such parameters as the specificity of the blocking antibodies, the stability of the cultured cells with respect to their sensitivity to, and dependency on, the mitogenic hormones for growth. It is planned to use the cultures for screening patients, including those having malignancies related to the pituitary.

Some of the transplantable lymphomas in the Nb rat are highly dependent on exogenous estrogen for growth; cells cultured from these lymphomas have shown a high dependency on PRL for growth in vitro. The estrogen dependent lymphomas can be encouraged to progress from hormonal dependency to autonomy by appropriate adjustment of the hormonal status of the host. Similarly, cultured cells from one of the lymphomas can be induced to progress from dependency on PRL for growth in vitro to a condition of autonomy. The relationship of the progression as observed in vivo to that found in the cultures will be investigated. Preliminary studies indicate the differences in the estrogen dependency of lymphoma lines in the animal can be correlated with differences in the PRL requirements in vitro of cells cultured from the lymphomas. (Text Abridged.)

166. HORMONES, THE CYTOSKELETON AND TUMOR CELL PROLIFERATION

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Androgen-responsive Shionogi 115 cells cultured in the presence of testosterone are fibroblastic in appearance and grow well in suspension culture. In the prolonged absence of testosterone they become androgen-unresponsive, change to an epithelial morphology and become unable to grow in suspension.

We have employed a variety of techniques to investigate the nature of cell adhesion and of cytoskeletal structure in androgen-responsive (plus-A) and unresponsive (minus-A) S115 cells. Interference reflection microscopy supported previous observations with the scanning electron microscope and showed that, whereas the plus-A cells had few areas of contact with the substratum and no focal adhesions, the minus-A cells had a greater area of contact and showed distinct focal adhesions. The most marked differences between the two cell types were found by fluorescence microscopy using an antibody raised against actin. Microfilament bundles and stress fibers could not be demonstrated in the plus-A cells which showed only a punctate distribution of actin. In contrast, the minus-A cells had large numbers of well-defined microfilament bundles, many of which extended across the cell. Both cell types showed a well developed system of microtubules with particular concentration in the perinuclear area. There was very little fibronectin on the surface of either cell type and the pattern on plus-A and minus-A cells was similar.

We concluded that androgens alter the growth rate of S115 cells partly by altering their substrate adhesive properties and anchorage dependence. These changes are closely related to the distribution of actin microfilament bundles and stress fibers.

167. STEROID CORRECTION OF THE CELL MATRIX IN NEOPLASIA

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Most notable among a variety of differences between normal and transformed cells is a loss of the cell matrix and association of matrix proteins with the cell membrane. Fibronectin and collagen are major components of the cell matrix and confer increased adhesion to cells. We have shown that low levels of glucocorticoid hormones (e.g., dexamethasone) can revert transformed cells towards normal (dex-R plus) leading to the development of an extracellular matrix of fibronectin and collagen. We will perform numerous studies to attempt to document the mechanism for this fundamental change induced by dexamethasone. We will ascertain whether dex can revert cells transformed in a variety of manners, and also tumors existing in man or animals. We will document whether dex modifies other phenotypic characteristics of cells and whether it is acting directly or functioning in some permissive role with other hormones or factors.

168. HORMONAL CONTROL OF CELL GROWTH AND DIFFERENTIATION

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This program, which has as its aim elucidation of mechanisms of hormone action at the molecular level, is being forwarded in several close-related areas: analysis of proximate interactions of hormones, both steroid and peptidal, at the outer surfaces of specific target cells; analysis of the secondary effects of such interactions and their immediate consequences in membrane perturbation and internalization; analyses of the lysosome-mediated transfer of signals arising from the above to other cellular compartments, including the cytoplasm and the nucleus; analyses of the contributions of recompartmentalized components of hormone-activated lysosomes upon subsequent transcriptional and translational events.

169. ENDOCRINE REGULATION OF MELANOMA CELL DIFFERENTIATION

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No summary has been provided to the Smithsonian Science Information Exchange.

170. INFLUENCE OF SOME REGIONS OF THE NERVOUS SYSTEM ON THE CELL MULTIPLICATION IN EXPERIMENTAL TUMORS

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Previous work showed that destruction of the tuberoinfundibular region of the hypothalamus in the rat is followed by a significant rise in the mitotic index of Yoshida ascites tumor cells. Subsequent to the destruction of the tuberoinfundibular region, the mitotic index, metaphasic index and cellularity (both total cell volume and total cell count of each tumor) of the Yoshida ascites tumor were studied. Both the mitotic and metaphasic indices were significantly higher than in the controls while cellularity was significantly reduced. The decreased cellularity of Yoshida tumor in rats that underwent destruction of the tuberoinfundibular region is thought to be due to an intensification of the processes of destruction of tumor cells. In another project we completed the study on growth and mitotic index of Yoshida ascites tumor in rats subjected to destruction of the tuberoinfundibular region of the hypothalamus plus hypophysectomy, in comparison with rats subjected to hypophysectomy only. The results confirmed that the rise in the mitotic index occurs after destruction of the tuberoinfundibular region even in the absence of the hypophysis. In other experiments we observed that after pinealectomy in the rat, total cell volume, mitotic and metaphasic indices of Yoshida ascites tumor are significantly increased. These results point out that the pineal gland has an inhibitory action on cell multiplication of Yoshida ascites tumor cells.

171. REGIONAL DIFFERENCES IN TUMOR GROWTH AND DEVELOPMENT

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The proposed research is directed at gaining a better understanding of the reason why tumors or normal tissues have a differential pattern of growth and development depending on where in the body they are located.

Specific experiments are designed to: 1) characterize further the nature of regional differences seen when tumor cells are inoculated into various portions of the body; 2) determine the nature of regional effects that might be exerted on growth and differentiation of normal tissues; and 3) determine what metabolic or developmentally significant processes might be correlated with the observed regional effects in the hope that this would permit establishment of causal connections between those processes and the observed regional differences.

Aside from the immediate practical implications resulting from the delineation of regional differences it is hoped that the experiments will provide basic insight into what may represent a critical control point regulating both normal and abnormal growth and development.

172. REGULATION OF DIFFERENTIATION DURING EARLY STAGES OF DEVELOPMENT (ALGAE)

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This is part of a broader project. A summary of this subproject is not available.

D. CYCLIC NUCLEOTIDES IN DIFFERENTIATION AND GROWTH

173. THE ROLE OF CYCLIC AMP IN THE REGULATION OF THE DIFFERENTIATION OF MUSCLE

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OBJECTIVE: The general aim of the project is an examination of the mechanism of action of cAMP in its role in the positive regulation of muscle differentiation. Typically, when cultured in vitro, embryonic myoblasts pass through an initial phase of proliferation which is followed by a cessation of growth, initiation of cell fusion and appearance of muscle-specific proteins. We have evidence which suggests that a transient rise in intracellular cAMP acts as a signal to initiate a sequence of covert events in the undifferentiated cells which results in the initiation of their terminal differentiation. The ability of prostaglandin E to provoke a precocious increase in both intracellular cAMP and myoblast fusion provided evidence for a causal connection between the two events. Furthermore, in a further study using inhibitors of prostaglandin (PG) synthesis, we were able to establish that PG production is an obligatory step in the differentiation of muscle in vitro. Preliminary experiments in which two other parameters of muscle differentiation, namely the receptor for acetylcholine (AchR) and the enzyme creatine phosphokinase (CPK), have been examined, suggest a similar involvement of the cyclic nucleotide in the regulation of their appearance in the differentiating cell. The increases in both of these parameters involve de novo synthesis, and in the case of AchR there is an associated increase in the number of receptors incorporated into the cell membrane. The increase in CPK activity is due in the main to the synthesis of a new muscle-specific isozymic form. The specific aim of the project is therefore to examine the nature of the involvement of cAMP in the appearance of these two proteins in more detail.

APPROACH: The experimental approach will involve the in vitro culture of primary chick myoblasts to examine their differentiation under control conditions and after a number of experimental manipulations designed to synchronize any cAMP-dependent changes in a part or all of the myoblast population. The latter will be achieved either by provoking the myoblasts to differentiate precociously with agents which raise intracellular cAMP or by initially blocking cell differentiation with inhibitors of PG synthesis. The rate of synthesis and incorporation of AchR moieties into the myoblast membranes will be assessed using 125I-labelled alpha-bungarotoxin. (Text Abridged.)

174. THE ROLE OF THE MULTIENZYMATIC SYSTEM OF CYCLIC NUCLEOTIDES IN THE GROWTH OF HUMAN AND EXPERIMENTAL BRAIN TUMORS

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Considering the importance of cyclic nucleotides (cAMP and cGMP) in modulating cellular growth (both in normal and neoplastic tissue) we have studied the metabolism of cyclic nucleotides in human tumors of different histological type and malignancy.

APPROACH: The project will deal with the following metabolic steps: a) Study of adenyl cyclase and of guanosyl cyclase as well as of phosphodiesterase (PDE) (acting on either nucleotide) in brain tumors. Data so far obtained indicate that both cyclase and PDE are lowered in neoplastic tissue. b) Study of the endogenous protein activator of PDE in brain tumors. c) Study of protein kinase in brain tumors. This part of the pro-

gram is now under investigation: preliminary results are briefly reported in the next paragraph.

Protein kinase (PK) activity has been investigated both in cytosol and in particulate fraction, in basal conditions and after stimulation with cyclic nucleotides. In basal derivation and malignancy. In neurinoma the basal PK is significantly stimulated in cytosol by both cyclic nucleotides (cAMP and cGMP). On the other hand, in glioblastoma and in astrocytoma, which are tumors of more pronounced malignancy, cGMP specifically induces a remarkable increase in PK activity, and this biochemical event is signficantly present only in the particulate fraction which is more important for the physiological activity of PK.

In tumoral tissues the ratio cAMP/cGMP dependent PK both in cytosol and in particulate fraction is decreased, in comparison with normal gray and white matter, indicating that tumors have a higher activity of the cGMP-dependent PK. It is important to note that only the ratio measured in the particulate fraction of glioblastoma and astrocytoma demonstrates a PK stimulation by cGMP higher than by cAMP, completely reverting the normal ratio pattern.

175. BIOCHEMICAL STUDIES OF DIFFERENTIATION IN CULTURED NORMAL AND NEOPLASTIC CELLS OF NEURAL ORIGIN

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OBJECTIVE: Dbc-AMP or agents that enhance cAMP formation induce morphological and biochemical differentiation of various tissue cultured cells. The nature of this process is studied in glioma (138 MG) and neuroblastoma (C 1300) cells and compared with the spontaneous differentiation of cultured astroblasts from brain hemispheres of newborn rats.

APPROACH: The cAMP-system is studied with respect to cAMP levels, activation of adenylate cyclase and phosphodiesterase activity during the differentiation process. Its relation to changes in high energy phosphates and ATPase activity is described.

Indirect immunofluorescence is used for visualization of changes in the cellular fibrillary structure, in particular the distribution of microtubules (MT) and glial intermediate filaments (GFA).

PROGRESS: The differentiation is partly linked to activation of the cAMP-system and is manifested in changes in energy metabolism, ATPase activity and reorganization of structural proteins.

176. CYCLIC AMP METABOLISM IN TRANSITIONAL EPITHELIUM

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The morphological time sequence of chemical carcinogenesis in transitional epithelium lining urinary bladder has been characterized in rat models. Due to the microscopically thin nature of the tissue in vivo it has been difficult to conduct physiological, biochemical, and morphological studies that precisely describe and compare the normal mechanisms of growth and differentiation to those of the carcinogenic process. An epithelial growth system which allows the growth, stratification, differentiation, and maintenance of homogeneous, adult Fischer-344 rat transitional epithelium in vitro has been developed. Using this system it is proposed: 1. to further define the requirements and factors governing growth, stratification, differentiation, and maintenance of normal tissue; 2. to determine the biological mechanism of carcinogenesis; and 3. to determine whether human transitional epithelium will grow and differentiate in the epithelial growth system. The normal tissue studies will include: continued development of a defined media; evaluation of porous growth surfaces; examination of the apparent differentiation-inducing effects of urine; determination of permeability characteristics; and resolution of the role of hormone-stimulated cyclic AMP metabolism in normal tissue. The carcinogenesis studies will include: determination of efficacious carcinogens; definition of morphological staging of in vitro carcinogenesis; assessment of the role of promoters; and analysis of a variety of events important in a two-stage mechanism of carcinogenesis (e.g., dose effects, sequence, reversibility-irreversibility; cell division, etc.). Tumorigenicity of in vitro transformed cell lines will be determined by back-transplantation. The human cell culture studies will utilize remnants of routine bladder punch-biopsies and will be carried out exactly as described for the rat epithelial growth system. These studies are being carried out to lay the foundation for determination of the molecular mechanism of chemical carcinogenesis of transitional epithelium.

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177. GROWTH REGULATION BY CYCLIC AMP

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This project is a study of animal cell growth regulation by cyclic adenosine monophosphate (cAMP). In S49 mouse lymphoma cells, cAMP slows cell growth by prolonging the G1 phase of the cell cycle. Studies with mutant cells reveal that cAMP acts in this system by activating protein kinase. Experiments are performed to study the kinetics of S49 cell cycle progression and its perturbation by cAMP. Synchronized cell populations are used to determine which effects of cAMP are independent of cell cycle position and which are not. Mutant cells are selected with specific defects in their ability to express the growth regulatory effect of cAMP. The intracellular substrates of cAMP-dependent protein kinase are being identified. These studies can result in a better understanding of the biochemical mechanisms by which cAMP regulates cell growth.

178. CYCLIC GMP SYSTEM IN CELL PROLIFERATION

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The objectives of the proposed research are to elucidate the functional roles of cyclic GMP-dependent protein kinase, its stimulatory modulator, and stimulatory modulator-requiring, cyclic nucleotide-independent protein kinase in normal and malignant cell proliferation. The experimental models to be employed are: Morris hepatomas (vs. normal livers) in rats, and regenerating livers (vs. sham-operated livers and normal livers). Attempts will be made to seek, characterize or identify possible changes in patterns, localizations and poperties or species of cyclic GMP-dependent protein kinase, its stimulatory modulator, and stimulatory modulator-requiring, cyclic nucleotide-independent protein kinase, as well as those of endogenous substrate proteins of the two enzymes, in cells undergoing proliferation. The above parameters will be compared to those obtained for cyclic AMP-dependent protein kinase and its inhibitory modulator.

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179. CYCLIC GMP AND CONTROL OF PROLIFERATION AND CELL FUNCTION

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The overall objective of the work is to define the biological importance of cyclic GMP and its related metabolic components. Based on recent observations in our laboratory that there is a selective effect of phosphotidylinositol (PI) relative to other phospholipids on the activities of the apparently soluble forms of guanylate cyclase and cyclic GMP-specific phosphodiesterase

from platelets and the concept that these presumed 'soluble' enzymes are associated with cell membranes in the native state, investigations are proposed to examine the interaction of these enzymes with membrane-related phospholipids. The investigations will include a study of the qualitative nature of the interaction by examining the effect of vesicles comprised of specific phospholipids. More quantitative aspects of the phospholipid interaction with the cyclase and phosphodiesterase will be carried out with phospholipid monolayers. This approach is intended to provide detailed information regarding the extent of and physical requirements for interaction of the cyclase and phosphodiesterase with the lipid-water interphase and the contribution of the physicochemical properties of the interphase to the catalytic process.

180. REGULATION OF CELL DIFFERENTIATION BY CYCLIC NUCLEOTIDES AND PROTEIN PHOSPHORYLATION

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The objective of this research project is to investigate the role of cyclic nucleotides and protein phosphorylation in signal transmission and regulation of cell differentiation in the cellular slime mold Dictyostelium discoideum. Our investigation will focus on (1) the characterization of plasma membrane-associated protein kinases and their endogenous substrates, and (2) the role of cAMP in the regulation of enzyme activities. Phosphorylation of a number of proteins is limited to discrete developmental stages and several of these phosphoproteins are found to associate with the cytoskeleton.

181. CYCLIC NUCLEOTIDES IN CANCER AND REGENERATING TISSUE

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The availability of antibodies that bind to the cyclic nucleotides with high specificity has made it possible to apply immunohistochemical techniques to the study of the localization of the cyclic nucleotide in tissues and cells. Of importance may be the distinct pattern of binding of the cyclic nucleotides to cellular organelles, and the relationship of this binding to the regulation of cellular growth. We are presently studying the binding patterns of cyclic nucleotides by utilizing immunohistochemical techniques in growing and regressing dimethylbenzanthracene induced mammary carcinomas. Regression is induced by either oophorectomy or administration of dibutyryl cyclic adenosine monophosphate. In addition we are quantitating cyclic nucleotide binding capacity with tritiated cyclic nucleotides, and determining cyclic nucleotide dependent protein kinase activity in different subcellular organelles in an attempt to correlate the quantitative data with our immunohistochemical observations.

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182. FUNCTION OF PROTEIN PHOSPHORYLATION IN MORPHOLOGICAL REVERSION

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In certain oncogenic, transformed fibroblastic cell lines evaluation of intracellular cyclic AMP results in the acquisition of normal cell growth and morphology. We have found that this 'reverse transformation' of Chinese hamster ovary (CHO) cells by dibutyryl cyclic AMP is accompanied by changes in the phosphorylation of specific cellular proteins. A 55,000 dalton protein (pp55) is phosphorylated, while a 20,000 dalton protein (pp20) is dephosphorylated. This research is designed to identify these phophoproteins and determine their function in the regulation of the transformed phenotype. The subcellular distribution of pp20 and pp550 and their association with the cytoskeleton will be investigated. Enzymological methods will be used to identify these proteins and establish whether pp20 is a myosin light chain. The protein kinase and phosphatase systems responsible for phosphorylation will be identified using cell free systems. Also using in vitro systems, the roles of cAMP, calcium and calmodulin (CDR) will be studied. The phosphorylation state of pp20 and pp22 will be correlated with the physiological parameters of reverse transformation: morphology, growth, substrate adhesion, the cell cycle and mitosis.

183. MOLECULAR MECHANISMS OF CYCLIC NUCLEOTIDE CONTROL OF CELL GROWTH

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The objective of the proposed studies is to define molecular mechanisms through which cyclic nucleotides and protein phosphorylation control cell proliferation. In order to understand regulation and function of the cyclic nucleotide effector systems, structures of G-kinase and A-kinase will be compared. Structure/function analysis of G-kinase will be directed toward cyclic nucleotide binding, protein regulatory, and catalytic kinase sites.

Purification of the protein product of the transforming gene of avian sarcoma viruses, pp60sor, will be initiated and investigation of substrates important for cell growth control will be sought. Interaction between cyclic nucleotide-dependent and pp60src kinase systems will be studied.

184. INTERRELATIONSHIP OF CYCLIC NUCLEOTIDE AND STEROID HORMONE IN GROWTH CONTROL

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Growth and regression of hormone-dependent mammary tumors are the function of the intracellular interplay between cAMP and estrogen. The interplay between cAMP and estrogen was reflected in the change in the activities of cAMP-dependent versus cGMP-dependent protein kinases in response to cAMP- or estrogen-stimulation of tumor slices in vitro. The interplay between cAMP and estrogen is also involved in the functional differentiation of mammary gland and in the tumorigenicity of carcinogen-induced mammary tumors.

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185. CYTOPLASMIC PHOSPHOPROTEINS AND THE REGULATION OF CELL GROWTH

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No summary has been provided to the Smithsonian Science Information Exchange.

186. CYCLIC AMP AND PROTEIN KINASES IN CELL GROWTH (MICE)

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This is part of a broader project. A summary of this subproject is not available.

187. CYCLIC NUCLEOTIDES AND LYMPHOCYTE PROLIFERATION

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This is part of a broader project. A summary of this subproject is not available.

E. POLYAMINE REGULATION OF GROWTH

188. RELATIONSHIP BETWEEN POLYAMINES AND CYCLIC NUCLEOTIDES - CONTROL MECHANISMS OF NORMAL AND NEOPLASTIC GROWTH

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Our previous observations indicate that polyamines, as spermine, spermidine and putrescine, at relatively low concentrations cause the decrease of cellular cAMP content, both in normal and neoplastic cell cultures. Considering the inverse relationship between cAMP concentration inside the cell and the rate of macromolecular synthesis and cell division, the effect of polyamines is consistent with their observed property to act as promoters of cell growth. Furthermore, results obtained by using specific inhibitors of polyamine synthesis, such as methylgliossal bis(guanylhydrazone) and -methylornithine, clearly indicate that cellular polyamine depletion is associated with cAMP accumulation and with a higher sensitivity of the cell to cAMP-mediated effectors, thus supporting the involvement of these biogenic amines in the control mechanism of cellular cAMP content. Preliminary experiments have indicated that in cultured cells, exogenous polyamines do not affect the release of cAMP but they reduce its intracellular content by increasing the rate of its degradation. Both soluble and particulate activities of cAMP-dependent phosphodiesterase (PDE) are elevated after treatment of the cells with the single amines. In this phase of research we will study the possible mechanism(s) by which polyamines affect PDE activity and in particular whether they exert their effects directly or indirectly through some endogenous factor that can activate PDE, such as calmodulin. Furthermore, since the activity of ornithine decarboxylase, the rate limiting enzyme in polyamine synthesis, is increased after cAMP accumulation, we will clarify whether this event is critical in inducing PDE activity by cAMP.

189. NEOPLASTIC GROWTH RATE AND POLYAMINE LEVELS IN TUMORAL TISSUES

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OBJECTIVE: The possibility to ascertain a correlation between neoplastic growth rate and polyamine levels in tumoral tissues is investigated. The finding of such a correlation may offer a test of biochemical malignancy. Moreover the study of polyamine levels in biological fluids of patients should be useful for earlier tumor diagnosis and for the evaluation of the efficacy of an antitumoral therapy.

APPROACH: Polyamine levels in tumoral tissues and biological fluids (blood serum, urine, lung washing, milk) are measured by gas chromatography, amino acid analyzer, and, as soon as possible, by HPLC.

PROGRESS: In larynx carcinoma (18 cases) obtained by laryngectomy, proteins, DNA, RNA, spermidine and spermine levels have been measured. Polyamines have been determined by gas-chromatography: RNA, DNA, spermidine and spermine levels are higher in tumoral tissues than in laryngeal tissue not invaded by tumor. Spermidine and spermine ratio is higher in tumoral than in normal tissue indicating a close correlation between spermidine/spermine ratio and malignancy seriousness.

190. POLYAMINES AS POSSIBLE MARKERS OF MALIGNANCY IN HEMATOLOGICAL TUMORS

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Polyamines are a naturally occurring, small cationic group, able to bind by electrostatic interactions to various macromolecules and biological structures thus modifying cellular processes. A very high concentration of these amines has been found in tissues with high RNA content and actively synthesizing proteins. In particular it is well known that polyamine concentrations are constantly increased in most tumors studied both experimentally and in humans. Naturally occurring spermine-binding proteins have been identified in rabbit and human serum, and a specific role has been proposed as a physiological conjugator for spermine molecules released from the cells.

Intracellular specific spermine binding proteins have been described in the cytosol of rat ventral prostate, and by us in the chick duodenal mucosa. Although the specific role of these intracellular conjugator proteins is not yet clear it is possible that they play a major role in the distribution and compartmentalization of polyamines within the cells.

It is our current interest to search for specific intracellular polyamine binder(s) in normal peripheral lymphocytes and in leukemia cells as a new approach to the study of polyamine physiology in normal and abnormal growth in humans.

191. FUNCTION OF POLYAMINES IN TUMOR CELL PROLIFERATION

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The polyamines putrescine, spermidine and spermine accumulate in rapidly growing cells and tissues before the synthesis of DNA begins. The purpose of this research is to study the biological function of polyamines in normal growth and tumor cell proliferation by using inhibitors of polyamine synthesis.

The inhibitory compounds are analogs of polyamine precursors or reaction products. The effects of new analogs will be tested on the activities of polyamine synthesizing enzymes and on the synthesis of macromolecules in cell cultures.

Our studies have shown that several ornithine analogs as well as diamino-propane and 5'-methylthioadenosine are potent inhibitors of polyamine synthesis, preventing the accumulation of polyamines and nucleic acids in cell lines derived from hamster tumors.

192. STUDY OF REGULATION OF CELL DIVISION - SEARCH FOR CELLULAR MUTANTS UNABLE TO SYNTHESIZE POLYAMINES

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OBJECTIVE: When cells start proliferating their polyamine content increases rapidly. The role of polyamines in the process of cellular proliferation has, however, remained obscure. Inhibitors of polyamine synthesis have been used in attempts to elucidate the mechanism of action of polyamines. It is, however, difficult to know whether the effect of inhibitors is based solely on their ability to block polyamine synthesis. Cellular mutants unable to synthesize polyamines would therefore be very useful in these studies. So far no such mutants have been obtained from animal cells. My objective is to isolate such mutants.

APPROACH: By mutagenesis and selective killing I have obtained mutants of hamster ovary CHO cells that show signs of deficient synthesis of polyamines. Now I will try to purify them and determine their ability to synthesize polyamines. If they turn out to be true 'polyamineless' mutants, I will compare the metabolism of these cells in the presence and in the absence of polyamines, hoping to gain some insight into the mechanism of action of polyamines.

193. ROLE OF POLYAMINES IN MAMMALIAN CELL PROLIFERATION

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Although high levels of polyamines, viz., putrescine, spermidine and spermine, are associated with rapid growth and cell division in eukaryotic systems, relatively little is known about their role in growth regulation. The recent discovery of specific inhibitors of polyamine biosyntheis, such as methyl gloxal bis (guanylhydrazone), alpha-methyl ornithine, and 1, 3-diaminopropane has opened up a new chapter in understanding the relationship between polyamine levels and other cellular functions. It is now well established that polyamines are essential for DNA synthesis in mammalian cells. Our preliminary studies with methyl glyoxal bis (guanylhydrazone) on HeLa and Chinese hamster ovary cells revealed that depletion of spermidine and spermine levels affect not only DNA synthesis but also chromosome condensation, cytokinesis, regulation of nuclear division and in vitro differentiation of mouse neuroblastoma cells. Under this project we propose to pursue these leads in a detailed and

systematic fashion using the techniques of cell fusion, premature chromosome condensation, scanning and transmission electron microscopy for ultrastructural studies of prematurely condensed chromosomes and immunoelectronmicroscopy to determine the role of polyamines in the structure and function of microfilaments. We also propose to study the molecular basis for the relationship between the depletion of polyamines and the inhibition of DNA synthesis. The role of polyamines in the regulation of nuclear division in normal and transformed cells will also be studied. The results of these studies may provide some clues as to how we could interrupt the division cycle of tumour cells by disrupting the biosynthesis of specific polyamines. A better understanding of the roles of polyamines at various points in the mammalian cell cycle may prove to be helpful in the control or treatment of human malignancies.

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194. MAMMALIAN POLYAMINE METABOLISM

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Polyamines are intimately linked to the growth of mammalian cells. Many changes in cellular metabolism induced by hormones and other agents are accompanied by marked increases in the rate of synthesis of putrescine and spermidine brought about by increases in the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase. The proposed experiments will extend knowledge of these enzymes (which have interesting regulatory properties and very rapid apparent rates of synthesis and degradation). These enzymes will be purified to homogeneity by affinity chromatography on pyridoxamine phosphate and methylglyoxal bis(guanylhydrazone) linked to Sepharose respectively. The purified enzymes will be used to raise specific antibodies to the enzymes. Specific irreversible inhibitors of both enzymes (namely alpha-difluoromethylornithine and 1,1'-((methylethanediylidene) -dinitrilo))- bis(e-amino-guanidine)) will be used to test the role of these enzymes in cell growth and differentiation. Labeled forms of these inhibitors will be used to measure specific binding to the enzyme and the number of enzyme molecules. Polyamines are known to be important in the growth of normal and malignant cells and knowledge of the regulation of polyamine synthesis and the effects of depleting cellular polyamines may enable the development of useful cancer chemotherapeutic agents either from existing inhibitors or by the synthesis of new polyamine antagonists.

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195. MECHANISMS OF GROWTH IN ANIMAL CELL CULTURES

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OBJECTIVE: Determine the relationship between the induction of ornithine decarboxylase (ODC) and DNA synthesis in HeLa cell cultures. Establish the relationship between the activity of ODC, polyamine concentrations and proliferation of HeLa cells. Characterize the regulatory function of the protein inhibitor, ODC antizyme, in DNA synthesis and proliferation of HeLa cells. Develop procedures to assay and biochemically characterize ODC antizyme.

APPROACH: HeLa cells will be maintained as monolayer stocks as liquid suspension cultures for experimental studies. ODC activity will be induced by the dilution of high-density, quiescent HeLa cell cultures with fresh growth medium. The time course of the induction of ODC, the synthesis of polyamines and the sythesis of DNA will be established in cells harvested at different intervals after addition of fesh medium to the cultures The inhibitory protein, ODC antizyme, will be induced by the addition of non-physiological diamines to cell cultures. The effect of the inhibition of ODC activity by ODC antizyme on DNA synthesis and proliferation of HeLa cells will be determined. Procedures for the purification and assay of the ODC antizyme will be developed. The results will increase knowledge of the regulation of proliferation in animal cells.

PROGRESS: Ornithine decarboxylase was stimulated in high-density HeLa cell cultures by dilution of or replacement of spent culture medium with fresh medium containing 10% horse serum. After stimulation, the enzyme activity reached a peak at 4-6 h, then rapidly declined to the low activity characteristic of quiescent cultures, where it remained during the remainder of the cell cycle. The stimulation of ornithine decarboxylase was eliminated by the addition of 0.5 MuM spermidine or spermine or 10 MuM putrescine to the HeLa cell cultures at the time of refeeding with fresh medium. Much higher concentrations (1 mM) of the non-physiological diamines, 1,3-diaminopropane or 1,3-diamino-2-hydroxypropane were required to eliminate the stimulation of ornithine decarboxylase in re-fed HeLa cell cultures. A heat-labile, non-diffusible inhibitor, ornithine decarboxylase antizyme, was induced in HeLa cells by the addition of exogenous diamines or polyamines. (Text Abridged.)

196. POLYAMINES, POLYAMINE ANTIMETABOLITES AND CELL GROWTH

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The role of the polyamines in cell growth and replication will be investigated by following changes in intracellular levels of the polyamines and in the activities of their biosynthetic enzymes, over the course of the cell cycle. Regulation of polyamine biosynthesis will be studied using inhibitors of nucleic acid and protein synthesis. The effect of polyamine antimetabolites on cell cycle traverse will be studied also. To provide a basis for the rational design of improved polyamine antimetabolites the structural requirements for binding to the active site of ornithine decarboxylase will be investigated. These quantitative structure-activity correlations will examine inhibition of ornithine decarboxylase by a series of structural analogs of ornithine. Finally, the potential applicability of polyamine antimetabolites to the chemotherapy of intracranial tumors will be explored using in vitro and in vivo colony forming efficiency assays and increased life span studies.

197. THE ROLE OF POLYAMINES IN THE DETERMINATION OF DNA TOPOLOGY IN BACTERIA

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OBJECTIVE: To clarify whether the involvement of polyamines in cell growth and proliferation, i.e., the physiology of polyamines, can be explained by a single, specific action on DNA gyrase.

APPROACH: By studying whether certain spermidine homologs show the same specificity in activating purified DNA gyrase as they show in restoring normal rates of growth, replication and macromolecule synthesis to polyamine-depleted E. coli mutants.

198. RESEARCH PROGRAM ON THE REGULATION OF CELL CYCLES OF NORMAL AND CANCEROUS MAMMARY CELLS

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The research programs to be pursued during the tenure of this award will involve primarily the study of agents which may regulate the cell cycles of normal and cancerous mammary

cells. Studies are planned in which the contributions of the polyamines, prostaglandins and cyclic nucleotides to the cell cycles of synchronized mammary cells will be determined. Cells to be studied include the human MCF-7 mammary cancer cells and cells from DMBA-induced tumors of rats.

199. PROTEIN KINASE SYSTEM IN RAPIDLY GROWING HEPATOMAS

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The applicant laboratory has recently identified a unique protein kinase system high levels of which are found in several rapidly proliferating mammalian cell systems. This protein kinase system is stimulated by polycations (e.g., spermine, spermidine, putrescine, polylysine, histones, etc.) and phosphorylates both tightly enzyme-bound endogenous proteins and non-bound low molecular weight endogenous proteins. Protein kinases are the well-established terminal enzymatic components of the system of cyclic nucleotides (cyclic AMP and cyclic GMP) which regulates processes of cellular growth and differentiation. Polyamines are also key regulators of cellular growth and differentiation, and they are elevated in rapidly proliferating mammalian cell systems. Initial comparsions of the polyamine 'responsive' protein kinase system in non-proliferating versus proliferating rat liver tissues show several major differences. It is proposed to 1) purify the polyamine stimulatable protein kinase from non-proliferating adult rat liver, from intermediate rate growing Morris hepatoma No. 7800, and from rapidly growing Morris hepatoma No. 3924A; 2) separate and compare catalytic moieties and enzyme-bound endogenous substrate proteins from each tissue by kinetic and physical protein methodologies; and 3) identify, isolate, and purify the non-enzyme-bound endogenous substrate proteins from each tissue. The proposed study will evaluate the role of protein kinase phosphorylation in the polyamine regulation of cellular growth. It is likely that the two cellular regulatory systems, cyclic nucleotides and polyamines, utilize a common enzyme system, a polyamine-responsive protein kinase. The proposed study will evaluate this possibility.

200. INTERFERON AND GROWTH IN MAMMALIAN CELLS

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Interferons are cellular proteins which inhibit DNA synthesis and division in normal and transformed animal cells. We propose that interferons can be used as experimental tools to study the control and regulatory processes involved in the proliferation of eukaryotic cells. Interferons display a selective inhibition of the synthesis of certain polypeptides in cells transiting from G1 to S phase. The nature of the above inhibition and the polypeptides involved will be examined to determine their role in initiation of cellular DNA synthesis. The bases for the inhibitory effect of interferons on ornithine decarboxylase, a key enzyme appearing in animal cells during their transit from normal to neoplastic growth, will be studied in detail. The control and regulation of polyamines and the involved biosynthetic enzymes will be investigated using intact cells as well as an 'in vitro' translational system for ornithine decarboxylase.

II. DIFFERENTIATION AND GROWTH IN SPECIFIC CELLS

A. NORMAL AND LEUKEMIC HEMATOPOIETIC CELL DIFFERENTIATION AND GROWTH

1. HEMATOPOIETIC STEM CELL DIFFERENTIATION AND PROLIFERATION

201. CELLULAR SPECIALIZATION AND REGULATORY MECHANISMS IN THE HEMOPOIETIC SYSTEM

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In 1977 we discovered that the largest erythroid colonies arising in our cultures of adult mouse bone marrow were not purely erythroid but contained, in addition, large numbers of megakaryocytes and occasionally cells of the granulocytic series. Subsequent experiments showed that these colonies are derived from pluripotent stem cells that undergo self-renewal (and produce spleen colony forming cells - CFU-S) concomitant with their differentiation during colony formation in vitro. Thus, we have now a powerful new system in which to investigate the control of stem cell behavior since effects can be evaluated at the single cell level under relatively controlled conditions. Objectives in the immediate future will be 1) to try and establish whether the acquisition of responsiveness to pathway-specific regulators (like epo) precedes of follows 'commitment' to that pathway; 2) to try and resolve the role played by extrinsic factors acting on stem cells, as opposed to factors intrinsic to the stem cell itself, in effecting self-renewal or restriction of differentiation potential. Preliminary studies strongly suggest that intrinsic variables are not only involved, but may actually determine the decision of extrinsically stimulated stem cells; and 3) to define more precisely molecular events that mediate (or regulate) stem cell decisions. The following lines of investigation are being followed: 1) Further characterization of cells that give rise to macroscopic mixed colonies in vitro - including assays for cells with granulopoietic, lymphopoietic and in vivo repopulating potential, as well as experiments to determine whether altered culture conditions (e.g., high vs low epo) affect the expression of these potentialities. 2) Attempts to determine how the process of stem cell self-renewal is regulated - triggering and delayed addition (of BEF/BPA) experiments will be used to test the extent to which factors (e.g., epo, testosterone, marrow cell conditioned media) that may activate resting stem cells into cycle, also initiate or support self-renewal. Serial replating experiments will be undertaken to determine whether self-renewal capacity decreases irreversibly and agents known to affect cyclic AMP and cyclic GMP levels, ion flux and other membrane properties will also be tested, alone and with glycoprotein regulators (e.g., epo, BEF/BPA) to further dissect the self-renewal mechanism.

202. STUDIES ON FACTORS INVOLVED IN DIFFERENTIATION AND PROLIFERATION OF HEMOPOIETIC CELLS IN NORMAL AND NEOPLASTIC CONDITIONS

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OBJECTIVE: To study - a) the possibility that molecules (factors) produced by neoplastic cells or by the interaction between normal and neoplastic cells might interfere in differentiation and/or proliferation of normal cells; b) the composition of these factors; and c) possible mechanism of action.

APPROACH: To study point a) - experiments to follow the formation of colonies by bone marrow and spleen from normal, leukemic and neoplastic mice with different Colony Stimulating Activity (CSA) and Conditioned Media (CM). To study point b) - purification and testing of CSA and/or CM which have shown some biological activity in point a) experiments.

203. CHARACTERIZATION AND INDUCTION OF DIFFERENTIATION OF HEMOPOIETIC CELL LINES

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The murine hemopoietic cell lines that we have established by virus infection of bone marrow cultures in vitro (Report 112) show distinct abilities to differentiate spontaneously. Our cell lines provide new model systems for studies on the differentiation of erythroid, granulocytic, megakaryocytic and B-lymphocyte lineages. The fact that some of the cell lines are nonleukemogenic suggests that it may be possible to consider these cells as in vitro analogues of the normals cells in vivo. This is particularly important in studies on differentiation as most other established cell lines of hemopoietic origin are leukemogenic and therefore may respond in an abnormal fashion to normal physiological inducers or may respond to treatments that do not

stimulate normal cells. Therefore, the comparisons of the effects of differentiation-inducing compounds on normal and leukemic cells may provide information on growth regulatory mechanisms. Moreover, studies are being undertaken to characterize the cells in regard to morphological, immunological, virological and biological criteria.

204. IMMUNOGENESIS FROM BONE MARROW CELLS

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This investigation is designed to study the differentiation of precursor lymphoid cells into clones of functionally discrete lymphocytes. Emphasis will be placed upon cell surface changes and regulatory events that direct early stages of precursor or stem cell differentiation into functional lymphoid cells, and the modulating influence of B cell development before and after antigen stimulation. Defined stem cell and lymphoid precursor populations and conditions for their molecular membrane interactions during differentiation will provide information for controlling lymphopoiesis for correcting deregulated cancerous states as lymphomas, myelomas and leukemias. Shed membrane molecular complexes serve to regulate development of functional lymphocytes. Developmental changes in surface membrane molecules will be correlated with differentiation of functionally discrete, biophysically purified classes of lymphocytes. These will be analytically compared and preparatively sorted for functional assays using both fluorescent activated and intrinsic scatter properties of laser excited cytometry. Cell surface characteristics will be analyzed with fluorochrome stains, liposomes, vesicles, and antibodies as molecular probes and markers which can be used to detect functional mechanisms of interaction that direct acquisition of immunological competence, its expression and control. Biochemical, immunochemical and biophysical analyses will parallel fluorescent cell sorter studies to provide a holistic approach to defining molecular events of membrane modulation during immogenesis. The objective of this proposal is to selectively engage control mechanisms responsible for differentiation stem cell progeny. Selective maturation, induction and interaction of functional lymphoid cells will eventually be used to repair deficiencies and provide immunological memory capacity for qualitative and quantitative development of the lymphocyte repertoire necessary for responses to neoplastic or infectious diseases.

205. LYMPHOKINE REGULATIONS OF BONE MARROW DIFFERENTIATION

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The pathway of differentiation of pluripotential bone marrow stem cells (BMSC) into fully mature functional thymocytes remains an enigma despite intense efforts to elucidate the maturation process. Most recently a major technical advance has made it possible to maintain BMSC in culture for extended periods. Preliminary experiments using a modification of the Dexter-type bone marrow culture system have indicated a regulatory role for soluble mediators released by lymphocytes in the commitment of hematopoietic stem cells into the lymphoid line of differentiation. These approaches make it possible to study this pathway of differentiation in greater detail.

In the present proposal extensive in vitro studies will be done to determine the kinetics and the optimal conditions for lymphokine control of BMSC proliferation of itself, i.e., self-renewal, as well as for the commitment to the lymphocyte differentiative pathway. Cells derived from cultures of BMSC stimulated by supernatants from cultures of alloreactive lymphocytes will be studied for expression of differentiation antigens and functional characteristics. Further maturation of cells derived from these cultures will be effected on monolayers of thymic stroma for study of terminal stages of T cell differentiation. Finally BMSC from lymphokine stimulated cultures will be used to prepare allogeneic chimeras which could then be examined to determine the function and specificity of their T cell populations.

206. THE ROLE OF HUMORAL AND MARROW STROMAL FACTORS IN THE REGULATION OF HEMATOPOIETIC STEM CELL PROLIFERATION

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The objective of this project is to study two different factors which influence hemopoietic stem cell (CFU-S) proliferation.

One factor is a putative CFUs stimulating factor (CFUs-SF) that is liberated into the blood as a result of CFU-S depletion. We will use an in-vivo culture system which we are currently developing for these studies. This system consists of placing marrow cells and isologous plasma in the presence of a source of hemopoietic stroma (longitudinally split femoral shaft) into dialysis membranes, which are then inserted into the peritoneal cavities of mice; and assaying for CFU-S in the contents at various times later. Both the source of plasma and of stroma can serve as variables in these experiments.

The second factor which we will study is the hemopoietic stroma which is required to support CFU-S proliferation (HS-P). This will be assayed, according to previously published methods, by determining the extent to which subcutaneously implanted femurs are able to support CFU-S growth. The extent and signficance of residual damage to HS-P function and to CFU-S proliferative ability will be determined after exposure to various amounts of radiotherapy and/or chemotherapy.

207. FUNCTIONAL DIVERSITY OF DIFFERENTIATING LYMPHOCYTES

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In order to immunologically correct aberrant regulation of lymphopoiesis which leads to cancers such as lymphomas, myelomas and leukemias, an understanding of normal regulatory events is needed. This project is designed to investigate changes which take place as stem cells differentiate into clones of functional lymphoid cell populations. The experimental approaches will make use of an in vitro system to elucidate the steps of maturation and the regulatory mechanisms which direct biophysically purified precursor cells in their development into functional helper, effector, memory and suppressor lymphocytes. Stem cell or precursor cell surface changes will be followed using membrane probes to correlate molecular modifications that arise during differentiation with lymphoid acquisition of functional capacity. Selective maturation and induction of functional lymphocyte populations in vitro could then be used to repair immunodeficiencies or to provide appropriate numbers of antigen-specific lymphocytes that are capable of restricting growth of neoplastic tissue in situ. Memory cells could be induced which would supply backup numbers of hyperactive effector or surveillant lymphocytes.

208. THYMIC MODIFICATION OF LEUKEMOGENESIS

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Many of the mechanisms for regulation of leukemic and normal hematopoietic differentiation remain to be elucidated. Pluripotent hematopoietic stem cells (HSC) proliferate and give rise to the circulating cells of the hematopoietic system. When malignancy occurs, leukemic cells take over the differentiative compartments of the bone marrow and eventually the leukemic process results in death. The presence of a functional thymus has been shown to be important for the resistance to RNA oncogenic viruses. We and others have evidence that the cells present in the thymus may interact with HSC to proliferate and differentiate into normal mature elements of the blood. Utilizing several experimental models of defective marrow proliferation, we intend to further explore the functional role of the thymus in the leukemic and normal differentiation of HSC. Thymosin, a humoral factor presently being used clinically for treatment of some cancers, will be examined for its potentiating effect on normal HSC differentiation. Development of methods in the model examined here might have broad application, useful for treatment of leukemia.

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209. PROLIFERATION AND DIFFERENTIATION OF STEM CELLS (MICE, RABBITS)

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This is part of a broader project. A summary of this subproject is not available.

210. PROLIFERATION OF HEMATOPOIETIC PRECURSORS IN ACUTE LEUKEMIA, PRELEUKEMIA (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

211. IN VITRO STUDIES ON LEUKEMIC CELL PROLIFERATION AND REGULATION

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This is part of a broader project. A summary of this subproject is not available.

212. REGULATION OF LEUKEMIC CELL PROLIFERATION

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This is part of a broader project. A summary of this subproject is not available.

2. ERYTHROCYTE DIFFERENTIATION AND PROLIFERATION

213. REGULATION OF ERYTHROID DIFFERENTIATION IN MURINE AND HUMAN LEUKEMIA AND THE GENETIC CONTROL OF HOST CELL RESPONSE TO LEUKEMOGENIC VIRUS

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We are investigating the mechanisms that regulate cell proliferation among early progenitors in the hemopoietic system, with the help of clonal cell culture methods. Our aim is to understand the operation of these mechanisms in normal individuals and how they become defective in the myeloproliferative disorders of mouse and man, so that improved strategies of management and prevention can be planned. We have found that a single gene Fv-2 (whose location is mapped on chromosome 9 in the mouse) controls both proliferation of an early progenitor cell (BFU-E) along the erythrocytic line of differentiation and susceptibility to Friend virus, which induces a primary polycythemia in mice. Very recently we have obtained evidence that BFU-E proliferation can be reversibly stopped through a macromolecular negative regulator from bone marrow whose production appears to be under the control of the same gene. We are presently trying to determine the nature and mechanism of action of this regulator and whether or not it also influences the growth of cells transformed by the polycythemia-inducing Friend virus.

214. THE MOLECULAR BASIS OF GROWTH CONTROL IN NORMAL AND TRANSFORMED HEMOPOIETIC CELL POPULATIONS

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OBJECTIVES: First, to gain a clearer understanding of the specific growth factors involved in regulating normal erythropoiesis; second, to determine which of these factors are ineffective in controlling transformed cell growth; and finally, to elucidate the molecular basis for altered response of transformed cells to normal growth regulators.

APPROACH: Human serum will be fractionated and fractions tested for effects on the terminal differentiation of erythroblasts. Those fractions with effects on normal human erythroblasts will be purified and tested on erythroblasts from patients with polycythemia vera and chronic myelogenous leukemia. Factors that are ineffective in controlling transformed cell growth will be used as probes to determine the molecular basis for altered responses of transformed cells to normal growth regulators.

PROGRESS: A new in vitro 59Fe suspension culture assay has been developed using adult mouse bone marrow cells. Using this assay, human serum has been shown to possess several large molecular weight factors, aside from erythropoietin and burst promoting activity, that both stimulate and inhibit erythropoiesis in vitro.

215. ERYTHROLEUKEMIA CELL DIFFERENTIATION

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The proposed project is a combined biochemical and genetic study on the expression of erythrocyte membrane functions during the induced differentiation of mouse erythroleukemia cells (MEL cells) in culture. Erythroid differentiation is promoted in these cells by addition of the inducing agent dimethylsulfoxide. The overall goals of the project are (1) to determine to what extent the MEL cell membrane comes to resemble that of the mature erythrocyte as a result of in vitro differentiation and (2) to determine whether the expression of red cell membrane functions is controlled separately or coordinately with expression of the genes coding for hemoglobin. Biochemical studies on the expression of red cell membrane functions are designed to determine whether red cell membrane proteins such as glycophorin and the band 3 protein (the anion channel) are synthesized in differentiating MEL cells and, if so, when they are synthesized relative to each other and relative to the time of hemoglobin synthesis. Complementary biochemical studies are intended to determine whether pre-existing MEL cell membrane proteins are modified or selectively degraded during the course of in vitro differentiation.

The genetic studies to be carried out in this project are devoted to determining whether expression of differentiated functions in MEL cells is controlled by one or by a series of several regulatory events. These studies will involve isolating genetic mutant or variant MEL cell lines which have lost the ability to synthesize hemoglobin because they are defective in the regulatory step(s) controlling hemoglobin gene expression. These 'white' clones will be examined for the synthesis of certain red cell membrane proteins, including spectrin and glycophorin. If differentiation is controlled in a single step then one expects that cells defective for the regulation of hemoglobin synthesis will also be unable to synthesize red cell membrane proteins.

216. REGULATION OF ERYTHROID DIFFERENTIATION IN NORMAL AND LEUKEMIC HEMATOPOIETIC CELLS

Deisseroth, A., Colbert, D., Hendrick, D., Bode, U., Fontana, J., Wright, D., Maniatis, T., Kan, Y., Stammatoyannopoulos, G., Weatherall, D., Rutherford, T., Tischfield, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Experimental Hematology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The genetic mechanisms governing hematopoietic differentiation in normal and leukemic erythroid and myeloid cells are being studied through use of cell lines which contain markers of human hematopoietic differentiation. Hybrid mouse erythroleukemia cell lines which contain the chromosome bearing the human alpha globin genes as the only human chromosome have been derived using human donor cells which differ with respect to their epigenotype, stages of development and genetic composition. A series of such hybrid cells, each containing human alpha globin genes from patients in whom non-deletion mutations have led to altered alpha globin gene expression, has been

constructed. The various steps of gene expression are being characterized using Southern and northern nucleic acid transfers as well as 2 dimensional gel chromotography of non-histone chromosomal proteins. Similar techniques are being applied to HL60, a continuously proliferating human promyelocytic leukemia cell line, in hopes of understanding the regulation of markers of monocyte and of myeloid differentiation in these human leukemia cells, as well as the process which governs commitment to normal maturation in normal cells which is defective in the HL60 cells.

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217. REGULATION OF CELLULAR PROLIFERATION IN NEOPLASTIC STATE

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The proposed study is directed toward evaluation of hematopoietic cellular proliferation in clinical disorders of blood associated with neoplasms, infection, polycythemia vera and other myeloproliferative disorders. The studies include: 1) Assay and characterization of regulatory factors (humoral or cell-mediated) present in these patients by the study of the in vivo effects of these factors in animals (mice and fetal sheep) as well as their influence on the proliferation and differentiation of animal and human bone marrow cells in vitro. 2) The evaluation of the proliferative characteristics of hematopoietic precursors by their response to physiologic regulators (erythropoietin etc.) and selected steroid metabolites in vitro. 3) The physical separation and characterization of erythroid precursors from bone marrow of patients with myeloproliferative disorders (and selected patients with anemias of infection and neoplasms) with capabilities of forming endogenous colonies in vitro and/or exerting inhibitory influences on erythroid colony formation by normal human marrow in vitro. Comparative studies will also be performed with fetal sheep hematopoietic cells. 4) Investigation of mechanisms underlying the progressive change in fetal sheep erythroid precursors from relative independence from erythropoietin to total dependence upon exogenous erythropoietin. 5) Development of a more meaningful assay for the myelostimulatory factor present in sera of some patients with myeloproliferative syndrome.

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218. MOLECULAR CONTROL OF FRIEND LEUKEMIA CELL MATURATION

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These studies are directed toward understanding the molecular basis of the control of cellular differentiation and its relationship to malignancy. Mouse erythroleukemia cells provide an excellent model system for this type of investigation since they undergo erythrodifferentiation to a hemoglobin-producing stage in tissue culture and in vivo. This differentiation is stimulated in vitro by dimethyl sulfoxide and other compounds. Many of these compounds are not structurally related. The induction of hemoglobin production by several of the inducers is accompanied by an increase in single-stranded breaks in DNA. Therefore the effects of several of the inducing compounds on DNA metabolism and structure in 'wild-type' and variant cell lines is being studied.

219. ERYTHROID DIFFERENTIATION IN FRIEND LEUKEMIA CELLS

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Three basic lines of investigation pertaining to the control of erythroid diffrentiation of murine erythroleukemia cells will be investigated. The molecular processes which are responsible for the commitment of these cells to terminal erythroid differentiation will be studied. The relationship between globin mRNA synthesis and commitment will be analyzed. The control of genes expressed during the terminal phase of the program will also be studied.

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220. MOLECULAR BIOLOGY OF HUMAN ERYTHROID DIFFERENTIATION

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No summary has been provided to the Smithsonian Science Information Exchange.

3. GRANULOCYTE AND MACROPHAGE DIFFERENTIATION AND PROLIFERATION

221. REGULATION OF CELL PROLIFERATION IN NORMAL AND LEUKEMIC BONE MARROW WITH SPECIAL REFERENCE TO CHEMOTHERAPY

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OBJECTIVE: a) Investigation of humoral regulators of hematopoietic cell proliferation. b) Cell kinetic response to chemotherapy in leukemia patients.

APPROACH: a) Cell cycle effects as well as target cell populations of partially purified extracts containing granul cyte chalone are investigated in different assay systems, including agar culture technique and diffusion chamber culture in vivo. b) The effect of different combination therapy schemes on cell cycle distribution of leukemia cells in peripheral blood are investigated with flow cytometry. This is done as a sequential study during the first five days of treatment of patients. The investigation is combined with cytochemical methods.

PROGRESS: a) Effects of granulocyte chalone extracts on the stem cell committed for myelopoiesis, as well as interaction with colony stimulating factor are studied. Cell cycle effects of the same extracts have been investigated in further detail. Some of this information has been published previously. Semipurified material of low molecular weight peptide from mature granulocyte has been studied in collaboration with Dr. W. Paukovits, Vienna. The factor interacts with myelopoietic colony formation in agar, and reversible inhibition can be obtained during a short exposure prior to plating in agar. b) With flow cytometry, an accumulation of cells with S-phase DNA content in peripheral blood is seen in patients with acute leukemia responding to combination chemotherapy (COAP and PRAP), and this is now under investigation in a larger number of patients.

222. REGULATION OF GRANULOPOIESIS

Shadduck, R. K., Waheed, A., Montefiore Hospital, Dept. of Medicine, *3459 5th Ave., Pittsburgh, Pennsylvania,* 15213, United States of America

The principal investigator has shown that neutropenia is associated with increased serum levels of colony stimulating factor (CSF). In vivo studies with various preparations of purified CSF suggest that this factor may have a granulopoietic effect in vivo. Moreover, the differentiation of granulocytes in diffusion chambers is markedly inhibited by administration of an antibody against CSF.

These ongoing studies will continue to purify large quantities of murine L-cell CSF and determine the effect of this factor

in normal and myelosuppressed mice. Recently, it has been possible to prepare high titer antibodies directed against pure CSF and to selectively fractionate these antibodies by affinity chromatographic techniques. These pure monospecific antibodies have shown partial inhibition of in vivo granulopoiesis.

The purified CSF and anti-CSF will be used to identify CSF producing cells by immunoperoxidase techniques. Moreover, the radioiodinated CSF will be used for binding studies for defining bone marrow stem cell cereptors. Further refinements of these techniques should allow for morphologic and functional characterization of the CSF responsive stem cells.

Radioiodinated GSF and high titer antiserum have been used to design a sensitive radioimmunoassay. This RIA will be used to determine the extent and type of CSF in murine serum and to evaluate the potential role of high molecular weight serum inhibitors.

BIBLIOGRAPHIC REFERENCES: Shadduck, R.K. and Waheed, A. Development of a radioimmunoassay for colony stimulating factor. Blood Cells 5:421-434, 1979. Zeigler, Z., Shadduck, R.K. and Winkelstein, A. Immune hemolytic anemia and thrombocytopenia secondary to quinidine: In vitro studies of the quinidine dependent platelet and red cell antibodies. Blood 53:396-402, 1979.

223. DIFFERENTIATION OF GRANULOCYTES AND MACROPHAGES

Burgess, A. W., Metcalf, D., Sparrow, L. G., Nicola, N. A., Cooper, P., Morstyn, G., Battye, F. L., Walter & Eliza Hall Inst. for Medical Research, Cancer Research Unit, *c/o Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia*

The objectives of this research are to analyze the structure-function relationships of granulocyte-macrophage colony stimulating factor (GM-CSF) and to probe the molecular responses of cells in the GM series when they are stimulated by GM-CSF. This regulator is known to be involved in combating infections and even tumor resistance through its involvement in granulocyte-macrophage production and activation. Hence, it is essential to analyze the structural features of GM-CSF which confer its biological activity and specificity. Micro amino acid sequence analysis of GM-CSF will be undertaken using thin layer peptide mapping, radiolabeled GM-CSF and high pressure liquid chromatography for the analysis and identification of the PTH amino acid. Large scale production methods for the low molecular weight GM-CSF (23K) will be investigated using cell hybridization techniques. It is intended to produce sufficient GM-CSF to prepare fluorescent derivatives for the investigation of the processing of target cell receptors and to aid in the separation of GM-CFC. GM development is also modulated by other regulators. In particular there are molecules in post endotoxin serum, distinguishable from the GM-CSF, which promote GM differentiation in myeloid leukemic cells. These differentiation factors and GM-CSF from thigh muscle CM will be purified for direct comparison with GM-CSF from mouse lung CM. Myeloid cells will be fractionated to provide pure cell populations for GM-CSF binding and activation studies. Using a double laser fluorescent activated cell sorter GM-CFC, immature myeloid cells, granulocytes and monocytes will be purified to greater than 95% homogeneity. Hybridoma immunoglobulins specific for immature myeloid cells, and FITC-labeled GM-CSF will be used to separate GM progenitors from precursor cells in the other series. Mature normal and leukemic granulocytes will be used as a source of the GM-CSF receptor. Activation by GM-CSF of specific nuclear, cytoplasmic or membrane protein biosynthesis in normal and leukemic GM cells will be analyzed using two dimensional gel electrophoresis. Protein changes will be related to the functional activation, differentiation or proliferation status of the cells.

224. REGULATION OF GRANULOCYTE AND MACROPHAGE PRODUCTION

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This proposal is directed towards: 1) Understanding the mechanism of action and physiological role of a subclass of the colony stimulating factors (CSF) that stimulates mononuclear phagocyte proliferation (CSF-1) and 2) using purified CSF and its specific antibody as probes to investigate the early events in hemopoiesis. Working with murine and human material, attempts will be made as often as possible to apply murine findings to the human system.

Studies on the mechanism of action will be carried out using biologically active 125I-CSF-1. The nature and distribution of CSF-1 binding cells in normal and leukemic murine and human cell populations will be examined. The mechanism of CSF-1 interaction with target cells, the relationship between target cell binding and responses and the characteristics of the CSF-1 'receptor' will be investigated. Parameters relating to blood cell differentiation and the mononuclear phagocytic system will be monitored in mice following injection of purified L cell CSF-1 and its specific antibody to determine the physiological role of CSF-1.

Highly specific antibody to both L cell and human urinary CSF-1 will be prepared. These antibodies will be used to examine the nature and distribution of CSF-1 releasing cells and to examine the genetic control of human CSF-1 production using human-mouse cell hybrids. Studies of the mechanism by which pluripotent stem cells give rise to the most immature CSF-1-responsive cells will be started.

225. PROLIFERATION AND DIFFERENTIATION OF HEMATOPOIETIC CELLS

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The proposed project is to study factors controlling the proliferation and maturation of hematopoietic stem cells in vivo and in vitro. The major emphasis is directed toward the study of in vitro proliferation of granulocyte-macrophage and megakaryocyte progenitor cells (CFU-GM, CFU-M) from the marrow of animals and man. Progenitor cells are cultured in a soft gel system (agar or methylcellulose) in the presence of a colony stimulating activity (CSA). In addition, cells will be cultured long-term in liquid suspension.

Studies will be directed toward the following: 1) To study the mechanisms of lithium induced granulocytosis, 2) to delineate factors controlling the proliferation of megakaryocytes and megakaryocyte progenitor cells, 3) to determine the virus-cell interaction in cytomegalovirus infection, 4) to determine the pattern of proliferation of hematopoietic progenitor cells in leukemia, preleukemia, and myelofibrosis.

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226. COMPARISONS OF NORMAL AND LEUKEMIC HEMOPOIETIC CELLS

Robinson, S. H., Glass, J., Page, P. L., Kruskall, M. S., Steinberg, H. N., Shulman, L. N., Harvard University, Boston Campus, Beth Israel Hospital, Dept. of Medicine, *330 Brookline Ave., Boston, Massachusetts,* 02215, United States of America

These studies are concerned with the mechanisms of abnormal cell growth and differentiation in malignant disorders of the hemopoietic system as approached through studies of the behavior of both normal and neoplastic hemopoietic cells. Ongoing work is concerned with the following related problems: the manner in which murine acute leukemic cells suppress normal hemopoiesis; the role played by different progenitor cells in the initiation and maintenance of steady-state granulopoiesis in diffusion chamber cultures of mouse bone marrow; the sequence of cellular events that characterizes chemotherapy-induced marrow failure and the efficacy of lithium carbonate in supporting granulopoiesis; the mechanism underlying the failure of macrophage growth to respond to factors altering granulocyte proliferation in diffusion chamber cultures of murine marrow; the in vivo factors supplied by host mice for plasma clot diffusion cultures which permit ready growth of early erythroid progenitor cells; studies of possible inhibitors of hemopoiesis produced by spleen; continuing in vitro studies of clinical disorders of granulocyte growth; and elucidation of the defect in iron transport that characterizes undifferentiated murine erythroleukemia cells.

227. THE STUDY OF PROLIFERATION AND DIFFERENTIATION OF MYELOID LEUKEMIA CELLS USING HUMAN LEUKEMIA LINES

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The specific objective of the project is to investigate the modulation of cell proliferation and differentiation in human myeloid leukemia using myeloid leukemia cell lines and selected somatic cell hybrids. I have found that the human acute myelogenous leukemia cell known as KG-1 responded to colony stimulating factor in liquid culture with an increased (3)H-thymidine labelling index and an increased rate of precursor incorporation into protein, RNA and DNA. CSF-stimulated thymidine incorporation by KG-1 cells in a dose-dependent fashion and dose-response curves were similar to those relating in vitro colony formation by KG cells or bone marrow precursors to various CSF concentrations. Thus the stimulation of DNA synthesis by KG-1 in the presence of CSF provides a convenient assay for human CSF. The effects of other hormones on human myeloid leukemia cell proliferation was also studied. We also looked at the effect of phorbol esters on myeloid differentiation. We found that leukemic cells blocked at the myeloblast and promyelocyte stage were able to differentiate into macrophage-like cells without cell division. Those cells blocked at an earlier stage of maturation were unable to differentiate into macrophage-like cells. I have also studied cell differentiation using somatic cell hybridization technique. We fused HL-60 (a promyelocyte leukemia line) with a Burkitt's lymphoma line and found that all myeloid characteristics were extinguished by the Burkitt's lymphoma genes. Further research in somatic hybridization and gene insertion is being pursued at this time.

228. DIFFERENTIATION AND FUNCTION OF HEMATOPOIETIC CELLS

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The goals of this research are: 1) To better understand the mechanisms by which cells, especially blood cells, differentiate from an immature to a functioning state, 2) to determine the levels of control of differentiation which are altered in poorly differentiated leukemic or other neoplastic cells.

Present studies involve characterizing changes in the protein gene products of HL-60 cells when stimulated to differentiate by chemical inducers, and also characterizing mRNA produced during that process.

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229. ROLE OF MEMBRANE LIPIDS IN MACROPHAGE DIFFERENTIATION

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The objective of the proposal is to further define the role of membrane lipids in macrophage differentiation. The initial approach was to quantitate membrane lipids at various stages of macrophage differentiation. However, preliminary data indicated that gross changes in lipid composition were not likely to explain the previously observed phenomena. In a related series of experiments it was discovered that macrophages express two pathways of plasmin formation and one pathway, a membrane or cell surface plasminogen activator, seems to reliably reflect the state of macrophage activation. Experiments are underway to assess the lipid requirements of this enzyme as a probe of lipid organization of macrophage plasma membrane. These experiments are being done in conjunction with other probes of lipid organization of macrophage membrane as the bulk of data now suggest that lipid organization of the membrane is a critical determinant of its biological activity.

4. LYMPHOCYTE DIFFERENTIATION AND PROLIFERATION

230. THE CONTROL OF LYMPHOCYTE PROLIFERATION AND DIFFERENTIATION

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OBJECTIVES: The objectives are to understand the mechanisms whereby leukocyte populations control their own proliferation and differentiation. The biochemical nature of intercellular signals, and their mode of action on lymphocytes, are of primary interest. Model systems for inducing cell-mediated toxicity toward cancer cells are to be developed. Understanding the controls operating to regulate the extent of proliferation, and the development of specialized responses like the immune reaction, may lead to a useful approach to controlling lymphocyte functions.

APPROACH: The present focus is on the characterization and mode of action of a particular lymphokine, which we have called co-stimulator, and which appears to be a second signal component of stimulating a number of lymphocyte responses. The effects of controlling the level of co-stimulator in model systems such as CTL and AFC generation are being studied. The relationship of this lymphokine to others described in the literature is to be investigated.

PROGRESS: Co-stimulator is a protein of MW 30,500, which is essential in generating mitogenic responses in murine thymocytes, in producing CTL to alloantigens or tumor antigens in vitro, and in obtaining AFCs from T cell-deprived populations of spleen cells. It is a product of T helper cells activated by 2 signals: a mitogen (or antigen) and a soluble macrophage product, lymphocte activating factor. Co-stimulator, in the presence of tumor antigen, stimulates a strong cytotoxic antitumor response when added to cultures of spleen cells from mice bearing normally fatal syngeneic tumors. These activated cytotoxic cells, injected into identical tumor-bearing mice, promote tumor clearance and enhance survival.

231. LYMPHOCYTE ONTOGENY IN CHILDHOOD ALL

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In a differentiating immune system substantial heterogeneity is expressed at the cellular level with respect to surface markers, metabolism, adequate triggers for differentiation and functions and their specificities. This heterogeneity seems mirrored in ALL, where one or many differentially expressed parameters may direct or signal course and prognosis. With the combination of new techniques, including the use of thymopoietic induction regimen, a number of assays for immunoregulatory functions, the development of monoclonal antibodies against surface glycoproteins and the study of purine metabolic pathways in leukemic cells, we may be able to dissect the developing immune system more comprehensively, derive new patient groupings in ALL and eventually recognize prognostic parameters in this leukemia. Our studies will focus on the active functions and the requirements for growth and differentiation of leukemic cells, in fact, consider them a functional part of the leukemic host. In addition to dissecting heterogeneity among leukemic patients, these studies promise new insights into some of the mechanisms which must direct response to therapy and its failure and they may delineate new strategies for therapy.

232. REGULATORY MECHANISM OF LYMPHOPOIESIS IN VITRO

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OBJECTIVE: Regulatory mechanism of T and B lymphocyte differentiation 'in vitro' under physiological and pathological conditions.

APPROACH: Human T lymphocytes isolated from peripheral blood can form colonies in semi-solid agar medium when stimulated with PHA-M and PWM. T cell subsets isolated by surface receptors from the Fc of IgG or IgM showed a remarkable difference in the cloning ability : TG cells develop few colonies; TM cells and T null cells accounted for the large

majority of the colonies formed by unfractionated T cells. A sharp suppression of colony formation occurred when positively selected TG cells were added to TG depleted populations.

The development of a technique for the in vitro cloning of normal T-lymphocytes can elucidate the control of growth and differentiation of lymphocytes and allow determination of the role of cell-to-cell interactions in the triggering of lymphocyte proliferation by mitogens and antigens. The changes in this control in lymphoproliferative diseases will be investigated.

233. LYMPHOCYTE DIFFERENTIATION IN CHILDHOOD LEUKEMIA

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This project seeks to investigate the nature of the cells of origin of acute childhood leukemias and the factors which govern their differentiation and proliferation. We plan to increasingly focus on the possibility that natural killer (NK) cells are committed pre-thymic T cell precursors which function as regulators in the hemopoietic system and as such mediate resistance to hemopoietic tumors. We will attempt to confirm that NK cells regulate EBV-induced B cell proliferation and examine by co-cultivation experiments the influence of various human T cell subpopulations on myelopoiesis and erythropoiesis. In addition we will attempt to determine the prognostic significance and therapeutic implications of all phenotypes defined by a panel of cell surface markers and to develop a mouse hybridoma which produces monoclonal antibody to human terminal transferase, an extremely useful leukemia cell marker.

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234. PROGRAMS OF NORMAL AND MALIGNANT LYMPHOCYTES

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Several years' study of T-cell and B-cell differentiation has led to the description of discrete sets of T-cells, and B-cells, and respective precursor populations. These can be distinguished by allotypic markers which characterize the surface phenotype of the respective sets. These sets differ in functional properties, and in the specificity of inducers that move them from one compartment of differentiation to the next. Another marker of lymphocyte differentiation is the template independent DNA polymerase, terminal deoxynucleotidyl transferase (TdT), which because of its unique biochemistry and tissue distribution, has been proposed as a mediator of generating the diversity of idiotypes in mature lymphocytes. We propose to examine the sequential and divergent differentiative steps of the lymphocyte lineage by determining the programming of expresson of TdT in thymus and bone marrow using biosynthetic and immunofluorescent assays for TdT and immunofluorescent and rosetting assays for allotypic markers. We propose to create tumor analogues for pre-T and pre-B cells in which TdT biosynthesis can be induced, by constructing hybridomas. These will be used to identify new allotypic markers and to study the molecular biology of TdT induction, both in terms of signals for induction, and alterations of cellular DNA upon induction.

We also propose to define the phenotype and resulting aberrant programming of lymphoid cells sets transformed by Abelson Murine Leukemia Virus, and by chemical carcinogen treatment of liquid bone marrow cultures. Such systems will create the tools to study the biochemistry of a program block in lymphocyte differentiation.

235. ION AND WATER METABOLISM IN NORMAL AND LEUKEMIC HUMAN LYMPHOCYTES

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These studies seek to define the critical factors controlling cell division in normal and leukemic lymphocytes, in anticipation of discovering differences that may be exploited in designing newer and more effective treatments of leukemia and other cancers. In the first year of this project these studies have defined the role of metabolism in controlling K, Na, Ca, and Mg metabolism, and the relationship of these ions to one another, by determining the effect of termperature variations and of ATP depletion on cell ion contents and on self-exchange of Na and K. The results suggest again that the conventional approach to the understanding of these processes is inadequate, and that cell ion contents and exchanges are determined primarily by association with a metabolite microenvironment that functions in a coherent fashion. Our ongoing studies are designed to test the hypothesis that the differences between normal and leukemic lymphocytes, and the differences in their ability to control cell replication, lie within a global defect in the metabolite microenvironment.

236. CONTROL OF LYMPHOCYTE DIFFERENTIATION BY SOLUBLE FACTORS

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This is part of a broader project. A summary of this subproject is not available.

237. INTERACTION WITH NORMAL LYMPHOID CELLS AS A GROWTH REQUIREMENT OF LYMPHOID TUMORS

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This is part of a broader project. A summary of this subproject is not available.

238. PROLIFERATION AND DIFFERENTIATION OF HUMAN T LYMPHOCYTES IN VITRO UNDER THE INFLUENCE OF STIMULATORY PROTEINS

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OBJECTIVES: Specific interactions between regulatory factors and their target cells are the basic mechanisms underlying regulation of normal hemopoietic cell differentiation. Leukemia-lymphoma and other hematological illnesses could be the results of disturbance of these specific cell-factor interactions.

APPROACH: To elucidate the mechanism of the cell-factor interaction, we used the differentiation of human T progenitor cells as the model system. T lymphocytes and their progenitor cells were grown long-term in a liquid culture in the presence of regulatory activity termed T lymphocyte growth stimulatory activity (TL-GSA) or grown clonally as T cell colonies in semi-solid medium. In the suspension culture, 6 kinds of functional progenies were detected. These included cytotoxic T cells, helper and suppressor T cells for B cell differentiation, responder for mixed leukocyte reaction, T cell colony forming unit (TL-CFU) and granulocyte macrophage colony stimulatory activity (GM-CSA) producing cells.

PROGRESS: Some of our findings are: 1) There is a common progenitor cell for human and B and T lymphocytes. 2) The factor-producing cells and factor-responding cells belong to the same clone. 3) The helper T progenitor cells in the suspension culture can generate at least two types of antigen-specific helper T cells (the antigens used were sheep erythrocyte and ovalbumin). 4) The cytotoxic T cells in the suspension and in the T cell colonies are capable of lysing at least three different cultured tumor cell lines. Since these cells are E positive and Fc negative cells, they are different from conventional natural killers. 5) There are two types of TL-CFU - one is the more mature TL-CFU which requires only PHA for colony formation; the less mature one requires an additional activity termed colony-promoting activity to convert them to sensitivity to PHA. Both types of TL-CFU can renew themselves extensively in culture according to a stochastic process and this process is regulated by an activity termed self-renewal enhancing activity (SREA). SREAs are nondialyzable glycoproteins with a mannoside terminal.

239. T CELL GROWTH AND DIFFERENTIATION IN LEUKEMIA

Chiao, J. W., Memorial Sloan Kettering Cancer Center, Dept. of Immunobiology & Developmental Biology, *1275 York Ave., New York, New York*, 10021, United States of America

We are undertaking an analysis of the potential for T cell differentiation and growth of precursor cells mainly in patients with acute lymphocytic leukemia. These cells have been shown to be incapable of differentiation in vivo. For this study, CM will be used to induce differentiation and development of the precursor cells associated with the null lymphocytes. Comparing the normal and patient T cell developmental sequence as delineated by emergence of cellular markers, subclasses and functions, we hope to understand the T cell developmental process and the nature of the abnormalities in leukemia. Employing the differentiation and proliferation assays, we will be able to analyze the stem cells, serum, growth and differentiation factors and their interactions in relation to the differentiation block and other abnormalities of the disease. We hope that this will facilitate a better definition of the leukemias and improve understanding of the regulation of hematopoiesis and the immune system in fundamental terms. We also aim at better detection and understanding of the factors involved in differentiation at the molecular level and specificities of its activity. This basic information will ultimately contribute to better treatment and perhaps prevention of the leukemias and other malignancies.

240. REGULATION OF T-CELL PROLIFERATION AND DIFFERENTIATION

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T-cell growth factor (TCGF) is a mitogenic protein released from mononuclear cells upon lectin or antigen stimulation. Detailed studies have shown that TCGF, which allows for the continuous proliferative culture of T-cells, acts as the mitogenic stimulus after lectin/antigen activation. The aims of the current project are to purify TCGF, develop a radiolabeled TCGF-cell binding assay, and produce monoclonal antibodies to TCGF and to the TCGF receptor. Other experimentation will be directed toward exploring the in vivo significance of TCGF and defining the mechanisms of TCGF production and action. It is hoped that these studies will provide new information and technical approaches which will lead to an increased understanding of the regulation of T-cells in both physiologic and pathophysiologic states.

BIBLIOGRAPHIC REFERENCES: P.E. Baker, S. Gillis, and K.A. Smith. Monoclonal cytolytic T-cell lines. J. Exp. Med. 149:273, 1979. S. Gillis, N.A. Union, P.E. Baker, and K.A. Smith. The in vitro generation and sustained culture of nude mouse cytolytic T-lymphocytes. J. Exp. Med. 149:1460, 1979.

241. DIFFERENTIATION OF IMMUNE SYSTEM - CELL SURFACE ANTIGENS

Kersey, J. H., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Laboratory Medicine & Pathology, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The overall objectives of this project continue to be related to differentiative pathways of lymphoid cells in humans. Emphasis is on T cell differentiation and bone marrow differentiation. Differentiation is studied in both normal and malignant cell populations, e.g., in leukemia and lymphoma.

The significance of these studies relates primarily to improved understanding of the immune system and its normal function as well as the abnormal function in immunodeficiencies, autoimmune disorders, and especially in leukemias and lymphomas.

242. STUDIES ON DIFFERENTIATION OF HUMAN T CELLS

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This is part of a broader project. A summary of this subproject is not available.

243. THE ANALYSIS OF HUMAN THYMUS-DEPENDENT DIFFERENTIATION

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This is part of a broader project. A summary of this subproject is not available.

244. ROLE OF THYMOSIN PEPTIDES IN T-CELL DIFFERENTIATION

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No summary has been provided to the Smithsonian Science Information Exchange.

245. T LYMPHOCYTE DIFFERENTIATION AND LEUKEMOGENESIS

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This is part of a broader project. A summary of this subproject is not available.

246. RECEPTORS FOR IMMUNOGLOBULIN ON HUMAN LYMPHOMA-LEUKEMIA

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Normal and neoplastic lymphocytes have surface membrane receptors for immunoglobulin. Receptors which are heavy chain class specific have been demonstrated with respect to IgG, IgM and IgA. In our preliminary experiments, we have shown that B lymphocytes will bind normal and neoplastic fluorescent labelled aggregated IgM and IgA. Utilizing these reagents, Ig receptors were sought on lymphoma-leukemia cells. Our results suggest that there may be correspondence between the heavy chain class specificity of surface membrane immunoglobulin and the corresponding immunoglobulin Fc receptor. By implication, therefore, one can construct a model of B cell differentiation involving a series of heavy chain specific immunoglobulin synthetic shifts corresponding to different levels of differentiation. Likewise, human lymphoid neoplasms which are generally B cell derived may be subdivided into potentially clinically useful subgroups according to these phenotypic markers.

We propose to study B cell differentiation pathways with respect to Ig receptors and SmIg in order to (1) characterize B cell differentiation, (2) delineate clinically important subgroups of patients with lymphoid malignancies who have differing prognoses based on marker phenotype. In order to do so, we will utilize 2 model systems. The first is a normal neonatal mouse spleen model which will provide information regarding the ontogeny of B lymphocyte receptors during fetal development. The second is the study of human lymphoid neoplasms. Fluorescent labelled reagents will be utilized and cells viewed under fluorescence microscopy. Cells will also be analyzed and sorted by the fluorescent activated cell sorter (FACS IV), particularly double fluorescent labelled cells. Heavy chain specific EA (Ig) complexes will be used to compare to the results obtained with Ig aggregates. Clinical information derived from patients with neoplasms studied for Ig receptors will be analyzed to determine whether there are differences in survival, response to treatment or other clinical paramaters which correspond to differing phenotypic profiles.

247. IMMUNOREGULATION OF MYELOMA CELL DIFFERENTIATION

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To facilitate the study of the cellular and molecular mechanisms of T cell-mediated regulation of B cell differentiation, we will use the DNP-specific IgA-secreting BALB/c mouse myeloma MOPC-315 as a monoclonal model of B cell differentiation. MOPC-315 cells differentiate from small, non-secretory lymphocytes (which predominate during early tumor growth) to

large IgA315-secreting plasmacytes during growth in normal BALB/c mice. That differentiation is sensitive to regulation by antigen-specific and idiotype-specific regulatory T lymphocytes. By centrifugal elutriation, MOPC-315 cell subpopulations will be separated by size and their functional activities (IgA secretion, stem cell activity, etc.) will be determined. Using these homogeneous MOPC-315 subpopulations, those surface markers and metabolic activities which are specific for each stage of B cell differentiation will be determined. I will also monitor how exposure of these neoplastic B cells to T cell regulatory factors and/or antigen affects the expression of the differentiation stage-specific markers. MOPC-315:MOPC-104E hybridomas which produce and secrete both IgA315 and IgM104E will be produced and used to probe how exposure to one regulatory stimulus affects the B cell's ability to respond to the opposite or same regulatory signal given later. These experiments will allow us to begin to understand how T cell regulatory signals are translated by the B lymphocyte.

248. IMMUNOREGULATION OF MURINE MYELOMA

Lynch, R. G., Milburn, G. L., Urnovitz, H. M., Washington University, School of Medicine, Dept. of Pathology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

This is a proposal to continue our studies of myeloma cell regulation. We previously demonstrated that the anti-TNP myeloma MOPC-315: 1) differentiates during in vivo growth; 2) is TNP-antigen 'sensitive' in the presence of carrier-specific regulatory T cells; and 3) is responsive to idiotype (Id315)-specific immunoregulatory signals. Those studies have recently been reviewed (Lynch et al., Immunol. Rev. 48:45-80, 1979). The major objective of the proposed studies is to understand the cellular and molecular mechanisms that mediate immunoregulation of myeloma cell growth, differentiation, and immunoglobulin expression. The proposed studies will emphasize an in vitro analysis of myeloma regulation and in addition to conventional effectors will utilize T and B cell hybridomas as monoclonal sources of idiotype (Id315)-specific effector cells and effector cell products. Issues of interest are: 1) the mechanism of action of Id315-specific, T cell-mediated secretory blockade of MOPC-315 cells; 2) the role of anti-Id315 antibodies in the regulation of Id315 expression and the induction of Id315-specific immunoregulatory cells; 3) the relative contributions of Id315-specific and TNP-antigen specific immunoregulators; 4) the developmental level of the myeloma cell at which a given immunoregulator operates; 5) identification and characterization of the immunoregulators; and 6) the biochemical events in the myeloma cell coincident and subsequent to immunoregulatory signal engagement. The proposed studies should provide knowledge that: 1) will further clarify our understanding of how normal B cells are regulated; 2) should provide insight into the organization of immunoregulatory curcuits and networks; and 3) may ultimately lead to an immunoregulatory approach to the control of malignant B cells in humans.

249. THE ROLE OF T-CELL FACTOR ON DIFFERENTIATION OF B CELLS

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This is part of a broader project. A summary of this subproject is not available.

250. ONTOGENIC DEVELOPMENT AND DIFFERENTIATION OF B-LYMPHOCYTES

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This is part of a broader project. A summary of this subproject is not available.

251. ANALYSIS OF B-CELL DIFFERENTIATION

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This is part of a broader project. A summary of this subproject is not available.

B. EMBRYONAL CARCINOMA CELL DIFFERENTIATION AND GROWTH

252. GROWTH AND DIFFERENTIATION OF HUMAN AND MOUSE TERATOCARCINOMA

Graham, C. F., Stern, P. L., Heath, J. K., Thompson, S., Gaunt, S. J., University of Oxford, Dept. of Zoology, *S. Parks Rd., Oxford OX2 6HE, England, United Kingdom*

OBJECTIVE: To understand the relationship between growth (in culture and in the organism) and differentiation of multipotential tumours.

APPROACH: The division and differentiation of mouse embryonal carcinoma cells is to be followed in clones to establish the role of cell selection in the loss of tumourigenicity as the cells differentiate. The factor requirement for growth of the tumor cells and their differentiated progeny will be analyzed and the susceptibility of the two cell types to immune responses studied. It is hoped to isolate multipotential stem cells from human teratoma and Wilms' tumour.

PROGRESS: The factor requirements of the stem cells are not understood but the differentiated cells can be induced to grow by epidermal growth factor, insulin, and glucocorticoids. The stem cells are very sensitive to lysis by natural killer cells while the differentiated cells are not.

253. CONTROL OF DIFFERENTIATION OF TERATOCARCINOMA CELLS IN CULTURE

McBurney, M. W., University of Ottawa, Dept. of Biology, *30 Somerset St. E., Ottawa, Ontario*, K1N 6N5, Canada

OBJECTIVES: When cultured under the appropriate conditions, malignant murine teratocarcinoma cells differentiate to give rise to a variety of simple tissue types, all of which are benign. Most of this project has to do with attempts to investigate this differentiation process with a view to understanding the developmental events which determine the fate of cell clones.

APPROACH: We intend to assess quantitatively the distribution of tissues formed during teratocarcinoma differentiation by means of a battery of assays for tissue-specific substances. Attempts will then be made to perturb the normal developmental program by allowing differentiation to proceed in the presence of certain drugs with known specific sites of action. It may be possible to correlate certain developmental anomalies with certain molecular perturbations and this should give us clues as to where to look for the important events involved with normal cytodifferentiation.

254. CELL INTERACTIONS IN THE DEVELOPMENT OF THE MOUSE EMBRYO AND IN THE DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS

Lacy, E., University of Oxford, Dept. of Zoology, *S. Parks Rd., Oxford OX2 6HE, England, United Kingdom*

The objective of the proposed research is to investigate the role of cell interactions in the development of the mouse embryo and in the differentiation of embryonal carcinoma (EC) cells. The inner cell mass (ICM) will be isolated from 4-day mouse blastocysts. The ICM will be dissociated into single cells, which will be grown apart in culture. At various time intervals, individual cells will be assayed for biochemical activities characteristic of extra-embryonic endoderm. The aim of the experiment is to determine whether the lack of cell interactions promotes ICM cells to differentiate into endoderm cells.

The behavior of isolated EC cells grown in monolayer culture will be recorded using time-lapse video equipment. At various times after the start of the transition from an EC cell to an endoderm-type cell, cells with known histories will be reacted with antibodies to endoderm specific gene products. This type of analysis will be used to determine whether there is a correlation between either the number of cell divisions or the number of cell contacts and the rate at which an individual EC cell develops into an endoderm-type cell. This experimental system will also be used to study the relationship between tumorigenicity and differentiation.

255. CELL INTERACTIONS IN TERATOCARCINOMA DIFFERENTIATION

Rosenstraus, M. J., Rutgers the State University of New Jersey, New Brunswick Campus, Douglass College, Dept. of Biological Sciences, *New Brunswick, New Jersey*, 08903, United States of America

The role of cell position and cell interactions in early mammalian embryogenesis will be studied in vitro, using clonal murine embryonal carcinoma (EC) cell lines as a model system. A hypothesis for the initial differentiation event, the formation of a 'rind' of endoderm on the outside of an EC cell aggregate, will be revealed by mixing experiments involving pluripotent and variant nullipotent (unable to differentiate) EC cells. Nullipotent (F-9 or Nulli-SSC1) and pluripotent (PSA-1) EC cells will be mixed to construct aggregates with specific geometries. The developmental fate (endoderm, mesoderm, ectoderm, EC or death) of the two cell types will be followed by autoradiography in histological sections. To accomplish this, subclones of pluripotent and nullipotent cells that differ in their ability to incorporate 3H hypoxanthine will be used. These studies will test the hypothesis that cells must be located on the outside of an aggregate in order to differentiate into endoderm. The hypothesis suggested by the first set of experiments can be further tested by similar experiments in which endodermal cells are aggregated with pluripotent and nullipotent EC cells. For future research aimed at detailing the steps in the differentiation pathway, developmental mutants of pluripotent EC cells will be isolated. These studies will help to define the mechanisms that lead to the differentiation and, consequently, the reversion of the malignant phenotype of EC cells.

256. MOUSE TERATOMA AND DERIVED CELL STUDIES

Chung, A. E., Braginski, J. E., Madhav, R., Lewis, M., University of Pittsburgh, School of Arts & Sciences, Dept. of Biological Sciences, *4200 5th Ave., Pittsburgh, Pennsylvania*, 15260, United States of America

The long-term objective of this project is to study the mechanism of differentiation of mouse embryonal carcinoma cells. The clonal cell lines PCC4-F, PCC4-aza-1, and PCao will be used for these studies. Emphasis will be placed on the in vitro differentiation of these cell lines. The specific approaches will be: (1) Examination of the influence of embryonal carcinoma cell cytoplasm on the gene expression in differentiated cells and the influence of the cytoplasm of differentiated cells on the multipotentiality of embryonal carcinoma cells, (2) comparison of the properties of the embryonal carcinoma cell with embryonal carcinoma derived lines PFHR-9 and M1536-B3, (3) development of an in vitro myogenic differentiation pathway and (4) exploration of the effect of a limited number of extrinsic factors on the differentiation of the embryonal carcinoma cell.

BIBLIOGRAPHIC REFERENCES: Properties of a Basement Membrane Related Glycoprotein Synthesized in Culture by a Mouse Embryonal Carcinoma Derived Cell Line. Albert E. Chung, Ronald Jaffe, Ian L. Freeman, Jean-Paul Vergnes, Janina E. Braginski, and Barry Carlin. Cell, 16, 227-287 (1979).

257. REGULATION OF CANCER CELL DEVELOPMENT

Brinster, R. L., University of Pennsylvania, School of Veterinary Medicine, Dept. of Animal Biology, *39th & Pine, Philadelphia, Pennsylvania*, 19104, United States of America

OBJECTIVE: Determine if the growth and development of cancer cells can be influenced by contact with cells of the embryo. Teratocarcinoma cells will be placed into mouse blastocysts, and their effect on subsequent development studies.

APPROACH: The techniques employed will be embryo culture, micromanipulation, autoradiography, skin transplantation, and electrophoresis.

258. STUDIES OF EMBRYONIC DIFFERENTIATION

Swartzendruber, D. E., University of California, Central Office, U.S. Dept. of Energy Los Alamos Scientific Lab., *P.O. Box 1663, Los Alamos, New Mexico*, 87545, United States of America

The objective of the task is to use teratocarcinoma cells, as a model of mammalian development, in conjunction with the techniques of flow cytometry to further define characteristics of differentiating cells and to help elucidate mechanisms of commitment to, and induction of, cellular differentiation. Flow cytometry will be used to: (1) quantitate specific structural, biochemical, antigenic and functional differences between teratocarcinoma stem cells and their differentiated progeny; (2) determine the proliferative capacities of teratocarcinoma cells in various differentiated states; (3) determine the relationship between cellular proliferation and cellular death by differentiation; (4) study the effects on differentiation of agents such as viruses, chemicals, biologically active compounds and toxic agents; and (5) identify and physically separate viable subpopulations of teratocarcinoma cells which are committed to various differentiative pathways, and thus, allowing studies on the molecular biology of events leading to the progressive restriction of developmental potential and specialization of function.

BIBLIOGRAPHIC REFERENCES: Cram, L.S., D.E. Swartzendruber and M.E. Wilder, Discrimination of Proliferating from Nonproliferating Cells and Single Cell Enzyme Measurements with Flow Systems, pp. 245-247 in Proceedings of the Second International Conference on the Automation of Cancer Cytology and Cell Image Analysis, N. Pressman and G.L. Weid (eds.), Tutorials of Cytology, Chicago, Il, 1979. Hoffman, R.A., and D.E. Swartzendruber, Electrical Impedance Analysis of Single Murine Teratocarcinoma Cells, Exp. Cell Res. 122: 426-429 (1979).

259. USES OF TERATOCARCINOMA STEM CELLS AS AN ALTERNATIVE TO THE NORMAL EMBRYO

Martin, G. R., University of California, San Francisco Campus, School of Medicine, Dept. of Anatomy, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

The proposed research involves studies of embryonal carcinoma cells, which are the stem cells of teratocarcinomas. These cells are pluripotent, that is they can form derivatives of all three primary embryonic germ layers. Certain mouse teratocarcinoma stem cell lines are capable of differentiation in vitro, in a manner which parallels normal embryogenesis. The major objective of the proposed research is to use such cell lines to gain new information about the processes of cellular determination and differentiation. In particular, we will study the process of endoderm formation by pluripotent embryonal carcinoma cells, and phenomenon of X-chromosome inactivation in vitro. Experiments will be performed using those cell lines which are already available, and new teratocarcinoma stem cell lines will also be isolated from tumors in a variety of murine inbred strains and from human teratoma tumors. We hope to obtain some euploid murine embryonal carcinoma cells lines which will then be used for blastocyst injections to generate new lines of mice and eventually to generate animal models for human genetic diseases. The obtained results should help us to gain a better understanding of the process of embryonic development as well as to elucidate the nature of the malignant stem cells and the factors which influence their ability to behave as normal embryo cells.

260. DIFFERENTIATION OF ENDODERM FROM TERATOCARCINOMA CELLS

Grainger, J. L., University of California, San Francisco Campus, Cancer Research Inst., Dept. of Anatomy, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Under the appropriate culture conditions, embryonal carcinoma cells differentiate into embyroid bodies, structures containing an inner core of undifferentiated stem cells surrounded by a layer of endoderm. These structures are analogous to the inner cell mass of early pre-implantation embryos. Three cell lines have been isolated that differ in the type and quantity of endodermal derivatives that form after the initial aggregation of embryonal stem cell. Using these cell lines and antibodies to a cell-specific marker CH1-fetoprotein, for visceral endoderm, or Reichert's membrane, for parietal endoderm, I plan to study the control of the differentiation of visceral and parietal endoderm under conditions that closely mimic the blastocoelic environment of the normal mouse embryo. By manipulating the ionic concentrations and the pH of the medium, it should be possible to study the control of the differentiation of endoderm as well as other terminally differentiated cell types.

261. CONTROLLED DIFFERENTIATION OF TEROTOMA CELLS

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This is part of a broader project. A summary of this subproject is not available.

III. CELL DIVISION AND PROLIFERATION

262. CELL PROLIFERATION IN EPITHELIAL TISSUES - GROWTH CONTROL, CARCINOGENESIS AND RESPONSE TO IRRADIATION AND CYTOTOXIC CHEMOTHERAPY

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OBJECTIVES: a) To identify and quantify stem cells in the small intestinal crypt. b) To analyze aspects of the control of cell proliferation in the intestine with special reference to the size of the functional compartment. c) An analysis of cell line specific growth control substances in the intestine. d) The evolution of clonogenic tumor cells during carcinogenesis in the intestine. e) The selectivity of cell death in proliferating and resting in vivo cell populations.

APPROACH: The approach will be mainly via orthodox cell kinetic techniques involving metaphase arrest, microdissection and autoradiography, and clonogenic assays involving microcolony analysis of the intestine and in the prostate gland.

263. REGULATION OF MITOSIS IN NORMAL AND TRANSFORMED CELLS

Sisken, J. E., University of Kentucky, School of Medicine, Dept. of Pathology/Physiology & Biophysics, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

Our long range goals are to understand the mechanisms of cell division and the changes which occur in these mechanisms when cells undergo neoplastic transformation. This study is based upon our observations that the duration of metaphase in transformed cells is longer than in normal cells and that the degree of prolongation may be related to the mode of transformation. Our aims are 1.) to determine the scope and generality of these findings, 2.) to determine the relationship between increase in metaphase duration and the increase in heteroploidy which occurs in transformed cells, 3.) to determine the relationship between increased metaphase durations and the increase in metaphase-to-prophase ratios observed in a number of tumors and 4.) to try to gain some insight into the control of mitotic events. Our methods will include quantitative analyses of time lapse cinemicrographic films of a variety of transformed and nontransformed cells in culture and an examination of the possibility of a correlation between metaphase duration and changes in karyotype of human and other mammalian cells. We will also attempt to gain a better understanding of the physiology of the metaphase stage and how it is altered in transformed cells by studying the response of such cells, as observed by time lapse cinemicrography, to agents which alter intracellular levels of calcium ions and cyclic AMP.

264. MEASUREMENT OF CELL KINETIC PARAMETERS OF HUMAN MALIGNANCIES BY IN VITRO AND LOCALIZED APPLICATION OF RADIOACTIVE DNA PRECURSORS

Jenkinson, I. S., Moore, F. J., McKerrell, P. J., St. Vincents Hospital, Dept. of Medical Physics, *Victoria St., Darlinghurst, N.S.W. 2010, Australia*

OBJECTIVE: To undertake further measurements of cell kinetics in accessible human pre-malignant lesions and malignancies such as basal cell and squamous cell carcinomata, and to correlate the measured kinetics with the morphology and growth rate of the lesions. The methods of measurement were selected and developed as being permissible and feasible for application to individual human tumors. They have been thoroughly tested in experimental animal tumors (Cell and Tissue Kinetics, 1975, 8:307-20) and in a pilot study on basal cell carcinomata.

APPROACH: Pre-malignant skin lesions together with squamous cell and basal cell carcinomata provide readily accessible abnormal cell population specimens containing a wide range of morphological patterns of cell growth. Cell production and loss rates are measured from very small biopsy specimens representative of various regions of the tumors. These are labelled with tritiated thymidine by incubating in oxygenated nutrient medium at 3 atmosphere pressure for 1 hour, and then micro-autoradiagraphed. From the morphological and spatial distribution of labelling index values together with net growth rate of the lesions, the cell production and loss rates are determined for various types of nodules and cell aggregates.

PROGRESS: Pilot measurements on basal cell carcinomata have yielded the same comparative trends in cell production and loss rates for a wide range of assumed doubling times. This was true whether variable or constant cell cycle times were assumed, or whether steady state or linear growth. The highest cell production and loss rates were in cell aggregates of adenoid form and in horn cysts, somewhat lower in small rounded nodules, and lower again in large multi-lobed nodules. Less convoluted nodules and large rounded nodules had lower production rates. Loss rates were also lower except for those with central necrosis which had very high loss rates. Large amorphous masses of cells had production and loss rates distributed over a wide range.

265. KINETIC CHARACTERISTICS OF HUMAN NEOPLASIA

Costa, A., Silvestrini, R., Daidone, M. G., Gentili, C., Rilke, F., Di Pietro, S., Salvadori, B., Istituto Nazionale per lo Studio e la Cura dei Tumori, Dept. of Experimental Oncology, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: 1. To study the correlation between the proliferative activity and clinical characteristics of human tumors and to define the clinical relevance of kinetic parameters to prognosis and to a correct evaluation of the efficacy of clinical trials. 2. To determine other biological characteristics (distribution of cells in the phases of cell cycle, ploidy and homogeneity of cell population) and to investigate their relation with proliferative activity and clinical features. Types of cancers being studied are non-Hodgkin's lymphomas (NHL), breast cancers, and testicular tumors.

APPROACH: Tumor material obtained by surgical excision is divided into two portions for histological examination and for kinetic (ARG) and cytofluorometric (CFM) analyses. ARG: Cell suspension from NHL and small fragments from breast and testis cancers are incubated at 37 degrees C for 1 hour with 3H-thymidine. Autoradiographies were performed with the stripping film technique. The labeling index (LI) is analyzed in relation to clinical characteristics. Its prognostic significance is checked by following the course of the disease. CFM: Cellular suspensions were fixed in 70 percent ethanol at 4 degrees C, treated wth RNase and stained in propidium iodide. The samples were measured by a flow cytofluorograph. Nuclei isolated from chicken erythrocytes (CEN) were used as an internal standard and DNA content was expressed as the ratio of the G0/1 peak of pathologic sample to that of CEN. The evaluation of DNA histograms was conducted with the mathematical model of Fried.

PROGRESS: NHL: The expanded series (from 66 to 88 patients) has confirmed the significant findings previously reported. LI is an important marker to be used for identifying prior to treatment the patients who could enter complete remission and have favorable survival. NHL (74 cases) showed an aneuploid DNA content in 61 percent of the cases. The abnormalities are mainly consistent with hyperdiploidy (91 percent). Among aneuploid tumors 24 percent were polyploid cell populations. A highly significant correlation (p less then 0.001) was found between the CFM S phase cells and ARG LI.

BREAST CANCER: The prognostic significance of LI of the primary tumor was assessed in 145 N-untreated patients. (Text Abridged.)

266. CYTOKINETIC BEHAVIOR OF NON-HODGKIN'S B-CELL LYMPHOMAS

Quaglino, D., Universita degli Studi di Modena, Inst. of Medical Clinics, *Via del Pozzo, c/o Policlinico, 41100 Modena, Italy*

With the purpose of obtaining useful prognostic information, we have investigated the cytokinetic behavior of 19 cases of non-Hodgkin's B cell lymphomas. In all cases diagnosis was established on the basis of histological, cytoimmunological and cytochemical data. The study, carried out with the aid of autoradiographic techniques, indicates that the various histological

subclasses are characterized by different metabolic and kinetic behaviors: the aggressiveness of the malignant lymphoma is closely related to the rate of growth of the lymphomatous cell populations, as assessed by the mitotic index and the labelling index with 3H-thymidine. The study of the synthesis and metabolism of RNA provides not only useful information on the energy requirements necessary for the neoplastic growth, but, when employed in association with immunohistochemical techniques for the detection of intracytoplasmic Ig synthesis, it defines more accurately the functional characteristics and the differentiation potentialities of the neoplastic populations in different cases.

267. CELL KINETIC STUDIES IN MYCOSIS FUNGOIDES AND SEZARY SYNDROME

Wantzin, G. L., Thomsen, K., Larsen, J. K., Finseninstitutet, Finsen Lab., Dept. of Dermatology, *49 Strandboulevarden, DK2100 Copenhagen, Denmark*

Cell kinetic studies in patients suffering from Mycosis fungoides and Sezary syndrome: Flow cytometric studies are performed on the dermal cell infiltrates, blood, and lymph nodes. A 3H-thymidine 5'-triphosphate labelling index is also performed. This index measures the fraction of a cell population with the potential for DNA synthesis or in active DNA synthesis. This index will be correlated with flow cytometric results. In addition, these cell kinetic parameters will be correlated with clinical data.

268. HISTOCHEMISTRY AND CELL KINETICS IN BONE TUMORS

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OBJECTIVE: Analysis of histochemistry and cell kinetics of bone tumors and tumor-like lesions in correlation with clinical prognosis.

APPROACH: Fresh tumor tissues are incubated in 3H-thymidine for 1 hour under oxygen; autoradiographic investigation of labeled nuclei in connection with histologically defined area. Histochemical investigation of different enzymes in connection with histologic and electron microscopic morphology.

PROGRESS: Fifteen osteosarcomas, chondrosarcomas, and other bone tumors differ from fibromas and other benign lesions in their histochemical enzyme patterns. Autoradiographic investigations of in vitro incubated tumors show different growth areas in tumors dependent on tumor types.

269. A STUDY OF CELLULAR PROLIFERATION AND DIFFERENTIATION IN NEOPLASTIC AND DYSPLASTIC LESIONS OF THE HUMAN BREAST

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OBJECTIVE: 1) To study the rate of cellular proliferation and alpha lactalbumin synthesis, which can be taken as a marker of functional differentiation of breast, in cases of mammary dysplasia and carcinoma. 2) To study the changes produced in alpha-lactalbumin synthesis vis-a-vis cellular proliferation under the influence of harmones, viz. estrogen, progesterone and prolactin, in cases of mammary dysplasia and carcinoma.

APPROACH: Cellular proliferation is measured in vitro using 3H thymidine and autoradiography. Alpha lactalbumin is quantitated in the tissue by immunosuppressive staining technique.

270. EXPERIMENTAL STUDIES ON HUMAN PROSTATIC CARCINOMA AND MOUSE PROSTATE

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Small pieces of human prostatic specimens are incubated with 3H-thymidine to assess DNA synthesis in the S phase of cell cycle. The ratio of active and inactive cells are determined in the acini from responsive and refractory cancer patients. Thus far results of autoradiographs show that about 15% of basal cells were synthesizing DNA. The results are providing a reliable index of tumor cell division and are useful in determining formation of anaplastic tumor, which is often refractory to estrogen therapy. In addition, using tissue culture techniques previously developed in my laboratory cell growth and mitotic index of radiation- and estrogen-treated prostatic cancer have been evaluated. Our preliminary data indicate that many subtleties are involved in the subcellular patterns of cancer cells in human prostatic carcinoma.

Ultrastructure of ventral prostate glands was studied in mice castrated for one through 60 days and for 11 and 17 months and in age-matched controls. We have described ultrastructural features of different populations of epithelial cells, their thymidine incorporation and freeze fracture chracteristics in castrates and normals. Our analysis of data has shown that the initial atrophy of prostate glands in castrates occurred by sloughing of the apical cytoplasm of columnar cells, autophagia of the cytoplasmic organelles as well as by sloughing of the individual cells into the acinar lumen. The cell loss by sloughing become infrequent in 7-to-21-day castrates. The cells sloughing increases secondarily in mice castrated for 21 to 37 days, but becomes minimal in castrates of 60 days and beyond. Our evidence suggests that the cell loss by sloughing is an active process in regression of prostate glands in castrates.

The effects of testosterone propionate on ventral prostate glands of castrated mice during the first 8 days of treatment were analyzed using ultrastructural and autoradiographic techniques.

271. INTERACTIONS OF DRUGS, RADIATION AND HORMONES

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Estrogen and other hormones are known to interact with both normal and malignant cells through specific receptor mechanisms and to effect changes in cell growth, division, and metabolism. The specific changes in cell cycle parameters, as well as the interaction of these hormones with radiation and cytotoxic drugs, are unknown. In this proposal we outline methods of examining the effects of estrogen and other hormones on viability as a function of cell cycle position, as well as the interaction of hormones and radiation and hormones and cytotoxic drugs on an in vitro human breast carcinoma line. To determine the in vitro kinetic parameters we will employ examination of growth curves and % labeled mitosis curves. Clonogenic survival curves and exponentially growing and plateau phase cultures will be employed to ascertain the effect of hormone, x-ray, and hormone, drug, and x-ray interactions in this cell line.

272. CYTOKINETIC AND METABOLIC STUDIES ON CYCLING RESUMPTION BY LECTIN-STIMULATED LYMPHOCYTES AND BY RESTING TUMOR CELLS

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OBJECTIVE: The aim of this research is to explicate the mechanism of recruitment into the phase of DNA synthesis (S phase) of normal and malignant cells after a period of resting. The rationale of this study is that recruitment into S is a crucial process in which analogies and differences between normal and cancer cells may be sought on which to base attempts in preventing tumor spreading as well as reactivation of dormant cancer.

APPROACH: The following cell populations were studied in their transition from the non-cycling to the cycling state in vitro: 1) Con A stimulated spleen lymphocytes from CBA mice; 2) radiation induced leukemia cells (RI cells), also obtained from CBA mice; 3) Yoshida AH130 ascites cells. The cytokinetic analysis of cycling resumption by these populations has been carried out by means of autoradiographic and radiometric techniques. The activities of fundamental metabolic pathways, such as respiraton, glycolysis, hexose monophosphate shunt, protein and RNA synthesis, are being studied as to their relevance to the cell transition from the non-cycling to the cycling state.

PROGRESS: The complex kinetics of mouse splenocyte response to Con A have been thoroughly defined. From this analysis it has emerged that in our system T lymphocytes respond to Con A stimulation with an intense proliferation, showing cell cycle parameters and growth kinetics very similar to those of RI cells in the same conditions. T blasts and leukemia

cells displayed similar rates of aerobic glycolysis. In all the populations listed above the G1-S transition was shown to depend on respiration. Studies carried out with ascites cells showed that this dependence does not rely on respiratory supply of ATP, which can be replaced by glycolytic ATP, but is linked to the electron flow through the respiratory chain.

273. CONTROL OF MACROMOLECULAR SYNTHESIS AND CELL DIVISION IN NORMAL AND TRANSFORMED CELLS

Alberghina, L., Popolo, L., Zippel, R., Mariani, L., Vanoni, M., Leonardi, M. C., Universita degli Studi di Milano, Cattedra Biochimica Comparata, *Via C. Saldini 50, 20133 Milan, Italy*

OBJECTIVE: The control of cell cycle progression is a central aspect for the understanding of the transformed phenotype.

APPROACH: a) Modeling studies: A dynamic model of the cell cycle is developed that takes into account the rates of ribosome and protein synthesis and the discontinuous events of DNA replication and cell division. The progression of the cell cycle is assumed to be controlled by critical cell mass acting on two threshold mechanisms. The model describes the growth kinetics and the macromolecular composition of populations of a known age distribution.

Three different patterns of cell cycle are possible which are similar to the experimentally observed cell cycle patterns. This model, with the insertion of a gaussian dstribution in the threshold mechanism(s) as a major source of variability, is able to describe quantitatively many kinetics and statistic properties of cell populations as the distribution of generation times, the alpha and beta curves, the cell size distribution, the correlations between sibling cells, etc.

b) Biochemical studies: The description of the macromolecular mechanisms of regulatory elements of the model will be pursued; (1) The cell sizing mechanism that controls DNA replication and (2) the mode of action of the competence factor(s). The platelet derived growth factor (PDGF) has been purified from human platelets. This factor in the presence of plasma induces DNA synthesis and cell proliferation in quiescent BALB 3T3 cells.

274. BIOMATHEMATICAL ANALYSIS OF NEOPLASTIC GROWTH PHENOMENA

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OBJECTIVE: The aim of the research is: a) To obtain a suitable formal description of cell kinetics phenomena for certain classes of tumors; b) To set up procedures to process experimental data in order to draw diagnostic and prognostic quantitative conclusions.

APPROACH: The methodologies of system theory, applied mathematics, and probability will be used to deduce mathematical models of certain phenomena of tumor growth and to subsequently validate and identify them against experimental data.

PROGRESS: a) Attention was focused on deterministic and stochastic models for the cell cycle, their structural properties, and their potentialities in concrete applications. b) Experimental cyclofluorimetric data were considered, in order to reconstruct the underlying DNA distribution in the various phases of the cell cycle.

275. BIOMATHEMATICAL ANALYSIS OF NEOPLASTIC GROWTH PHENOMENA

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OBJECTIVE: The aim of the research is a) To obtain a suitable formal description of cell kinetics phenomena for certain classes of tumors; b) To set up procedures to process experimental data in order to draw diagnostic and prognostic quantitative conclusions.

APPROACH: The methodologies of system theory, applied mathematics, and probability will be used to deduce mathematical models of certain phenomena of tumor growth and to subsequently validate and identify them against experimental data.

PROGRESS: a) Attention was focused on deterministic and stochastic models for the cell cycle, their structural properties, and their potentialities in concrete applications. b) Experimental cyclofluorimetric data were considered, in order to reconstruct the underlying DNA distribution in the various phases of the cell cycle.

276. INTERACTION BETWEEN CARCINOGENS AND INFORMATIVE STRUCTURES - STUDY ON THE REGULATION OF CELL PROLIFERATION

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OBJECTIVE: To study the regulation of cell proliferation and, mainly, the G0-to-G1 transition in in vivo-in vitro systems.

APPROACH: 3H-Thymidine uptake by purified DNA of isolated rat liver perfused with human RBC is determined at different times after the start of the perfusion. The effect of specific inhibitory substances (chalones) from normal liver is also investigated.

PROGRESS: 3H-Thymidine uptake by isolated perfused rat liver was studied. A high DNA synthesis starting 3, 4.5, 6 hr after perfusion had begun was observed. 3H-Thymidine uptake by DNA from liver nuclei was determined after purification by CsCl sedimentation. In order to determine a possible inhibition of mitotic cycle by rat liver-chalone(s), further experiments are now being conducted.

277. CONTROL OF PROLIFERATION OF NORMAL AND NEOPLASTIC CELLS IN VITRO

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OBJECTIVE: To investigate the proliferative properties of normal and neoplastic cells grown in vitro.

APPROACH: Combined autoradiographic, cytophotometric and time-lapse photographic techniques are used to investigate cellular growth properties.

PROGRESS: The mitogenic effect of short alkaline treatment on serum-starved cells has been described. The relationship with glutamine in the regulation of cell growth has been investigated.

A cell-cycle analysis of 3T3-cells exposed to short periods of serum starvation has been carried out. Studies using antibodies to DNA-nuclear protein complexes to reveal nuclear changes that accompany growth activation have been completed.

278. STUDIES OF MAMMARY GLAND REFRACTORINESS TO CANCER

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Breast cancer risk in women is markedly reduced by an early pregnancy and lactation. Using an experimental model we have demonstrated that the susceptibility of rats to carcinogenesis decreases with aging and is nullified when pregnancy and lactation occur prior to carcinogen administration. We have shown that the susceptibility of the mammary gland to carcinogenesis is influenced by variables such as the degree of differentiation of the gland, rate of DNA synthesis and cell proliferation, and variations in cell kinetics. The above-mentioned facts were substantiated by determining the length of the cell cycle (Tc) and of each of the cell cycle phases, and the growth fraction (GF) of the epithelial cells lining the mammary gland structures.

Our results indicate that in the mammary gland of young virgin rats the various structures developed as a consequence of progressive differentiation of the gland, have markedly different GF, which is highest in the least differentiated structure, the TEB, decreasing progressively in the more differentiated structures, TD and AB. The more undifferentiated structures have a shorter Tc, while in the more differentiated ones a progressive lengthening of Tc, due mainly to a lengthening of G1, is observed. These differences between the more differentiated and the undifferentiated are accentuated with aging. Complete differentiation of the mammary gland as a consequence of pregnancy and lactation eliminates the undifferentiated structures, with a concomitant decrease in GF and more marked lengthening of Tc. Pregnancy induces in the mammary gland two basic

changes in cell kinetics parameters: one of them is the increase in the size of the G0 compartment, and the second change is the lengthening of Tc at the expense of G1.

Our next objective is to determine whether these differences in cell kinetics are associated with a different DNA-repair capacity of the cell in susceptible and non-susceptible animals.

279. DETAILED CELL KINETIC ANALYSES OF HUMAN NEUROBLASTOMA

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The overall goal of this project is to increase our basic knowledge of the biology of cell replication in human neuroblastoma. Two models will be studied: the first consists of several human neuroblastoma lines in vitro and the second consists of these neuroblastoma lines grown in solid tumor form in the immunodeficient nude mouse. These tumors will be examined for synchrony, diurnal variations, and changes in proliferative characteristics with increasing size. The classic cell kinetics measurements, such as labelling and mitotic indices, and fraction-labelled mitoses curves, will be performed to determine baseline cell cycle times and parameters. Several newer techniques will be used also. First, flow cytofluorometry will be used to study overall changes in DNA distributions wthin the tumors. Combined tritiated thymidine autoradiography and DNA cytofluorometry will be used to provide independent measurements of DNA synthesis status and cell cycle position to study the kinetic characteristics of proliferating and non-proliferating cells. These studies will provide a detailed analysis of the cell kinetics of untreated neuroblastoma in order to provide a baseline with which treatment can be rationally formulated and against which therapeutic efforts can be objectively compared.

BIBLIOGRAPHIC REFERENCES: Neely, J.E.: Constancy of Cell Kinetic Parameters in a Human Neuroblastoma Line in the Nude Mouse, in Recent Advances in Neuroblastoma Research (A.E. Evans, ed.), 1980, in press.

280. PRIMARY EVENTS IN REGULATING CELL MULTIPLICATION

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We shall determine whether the slowdown of cell multiplication in Mg(plus plus) derived 3T3 cell cultures occurs in the G-1 period of the cell cycle or elsewhere, using autoradiographic and flow cytometric techniques. The results will be correlated with changes in the intracellular and surface bound concentrations of the 4 major cations. Similar studies will be done with a spontaneously transformed variant of 3T3 cells which requires much less Mg(plus plus) for maximal growth. Since the transformed cells also assume normal morphology in low Mg(plus plus) other properties of the cells in low Mg(plus plus) such as anchorage independent growth will be studied. Divalent cation ionophores will be used to make rapid changes in concentration of intracellular Mg(plus plus) and Ca(plus plus) and the effects of these changes on uridine phosphorylation will be determined. The aim here is to evaluate rigorously the role of divalent cations in a relatively simple early response of cells to external stimuli. This will require detailed kinetic comparisons of changes in intracellular ions, uridine uptake, ion effects on uridine phosphorylation in cell extracts and a search for other possible effectors of the reaction.

BIBLIOGRAPHIC REFERENCES: Rubin, A. Harry, and Daniel F. Bowen-Pope. Coordinate Control of Balb/c3T3 Cell Survival and Multiplication by Serum or Calcium Pyrophosphate Complexes. J. Cell. Physiol. 98, 81-94, 1979. Bowen-Pope, D.F., Vidair, C., Sanui, H. and A. H. Rubin. Separate Roles for Calcium and Magnesium in their Synergistic Effect on Uridine Uptake by Cultured Cells: Significance for Growth Control. Proc. Natl. Acad. Sci. 76, 1308-1312, 1979.

281. STUDY OF THE GROWTH KINETICS OF EPIDERMIS, SEBACEOUS GLANDS AND HUMAN MAMMARY TUMORS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

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OBJECTIVE: To determine the different cellular mechanisms involved in the response to endogenous regulators of cell proliferation of epidermal cells in: 1) short-term skin organ culture, 2) long-term cultures of pure dissociated epidermal cells and 3) the in vivo situation. A comparison will be made with sebaceous and mammary glands.

APPROACH: Mouse pinna epidermis and sebaceous glands will be studied in vivo using the technique of Thornley, Spargo and Laurence (1977, Brit. J. Derm. 97, 11023) in short-term organ culture already established (Spargo, submitted Ph. D. thesis, University of London); and in epidermal cells established through 14 generations in vitro by Dr. P.A. Riley, (Dept. of Biochemical Pathology, University College Hospital Medical School, London) with whom this work will be performed. Short-term organ cultures of human mammary glands will be used for comparison with their homologue, the sebaceous glands. The results will be obtained using standard autoradiography and scintillation counting techniques.

Analysis of the effects of time (circadian rhythm, time in culture, etc.), stress, stress hormones, beta-adrenergic agents and purified pig skin fractions on the cell kinetics will be conducted. The results should identify the existing similarities and differences between populations of epidermal cells in the three systems. The nucleotide pools and enzyme activity e.g., thymidine kinase, ornithine decarboxylase and adenylate cyclase will be analyzed, and where possible, these measurements will be related to the cell cycle events.

It is proposed to ascertain whether the 72-81 percent ethanol fraction of pig skin which is known to contain sebaceous gland chalone also inhibits the cell proliferation of its homologue, the mammary gland. It is necessary to test this in vitro since hormonal effects mask the activity of normal tissue extracts in vivo.

Lastly it is proposed to use these systems to study skin carcinogens e.g., application of dimethyl benzanthracene and phorbol esters at various stages of the cell cycle and in different physiological conditions.

282. HUMAN EPIDERMAL CELL PROLIFERATION STUDIES BY SINGLE CELL DNA FLOW CYTOMETRY ON NORMAL AND ABNORMAL KIN (ABBREV)

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OBJECTIVE: The aim of the study is to extend the use of a technique for single cell DNA measurements by flow cytometry successfully used in the hamster cheek pouch to studies on normal and abnormal human epidermis, e.g., premalignant and malignant conditions and psoriasis.

APPROACH: The reproducibility of the technique's measurements under standardized conditions is evaluated as well as the importance of inevitable fluctuation in several steps of the biopsy and preparation procedure.

The effect on the measured cell kinetics parameters in normal human epidermis of age, sex, anatomical region and time of the day as well as the interindividual variability is estimated.

The usefulness of the technique for clinical and therapeutical studies on cell proliferation in unaffected and affected skin in individuals suffering from solar or arsenical keratosis, Mb. Bowen, basal and squamous cell carcinomas and psoriasis should be evaluated.

PROGRESS: The reproducibility of the measurements is estimated to be sufficient for the purposes in question; the variability in results from 14 samples of normal abdominal skin of a female aged 26 expressed as a percentage of viable cells, being 3.0 % plus or minus 0.6 (SD) for the S-fraction and 1.1 % plus or minus 0.1 (SD) for the G2 plus M-fraction. Estimations of the influence of sex, age, time of day and anatomical region have shown certain variations depending less on region than on sex and age, while only a minor influence on cell kinetics parameters by the time of day was noted.

283. DIFFERENTIATION AND STROMA-INDUCTION IN NEURAL TUMORS

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The object of this research is to study, in in vitro systems established in this laboratory and in an appropriate animal tumor model, some of the phenomena pertaining to cell differentiation, tumor maturation and stroma-induction, as expressed morphologically, biochemically and immunologically, in neoplasms of the central and peripheral nervous system. The tumors investigated include human neoplasms; experimental rat tumors obtained following transplacental ethylnitrosourea (ENU); an established glial rat tumor cell line (C-6); and the neuroepithelial component of a transplantable mouse testicular teratoma (OTT-6050) maintained in syngeneic animals in ascitic and in solid forms.

Two main approaches are used: 1. The establishment of suitable organotypic culture systems that permit the sequential study of differentiation by morphologic, immunologic and immunoradiometric methods. Autoradiographic studies are being undertaken to determine the kinetics parameters applicable to these cultures, particularly in reference to the growth fraction that can be estimated in the steady-state regions of the explants.

2. The biological properties and in vivo and in vitro behavior and manipulation of the mouse transplantable teratomas as a model for neuroepithelial neoplastic differentiation. The interesting aspect of this model lies in the model's applicability to the study of embryonal central nervous system tumors in man. A long-term objective is the production of a pure experimental tumor in which differentiation is restricted to neuroepithelial derivatives. Markers of differentiation include those concerned with gliofibrillogenesis (GFA protein), cell surface membrane antigens specific for neuroepithelial cells, melanin production in association with primitive neuroepithelium, and the determination and quantitation of biogenic amines by high-sensitivity microspectrofluorometry.

284. CELL KINETICS OF NORMAL AND NEOPLASTIC HUMAN HEMATOPOIETIC AND IMMUNOLOGICALLY ACTIVE CELLS

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OBJECTIVE: Development of methods for combined immunological, cytochemical, and cytokinetic studies on single bone marrow and peripheral blood lymphoid cells. Investigation of children with ALL and lymphoma before, during and after treamtent.

APPROACH: Ficoll isolation lymphoid cells are pulse-labeled in vitro with radioactive specific DNA precursors; exposed to fluorescent, specific antibodies and sheep erythrocytes; centrifuged on slides; mapped with a computer-controlled scanning microscope fluorometer; evaluated for fluorescence; stained for unspecific acid esterases; evaluated for morphology and esterase reaction; processed radioautographically and evaluated for nuclear labeling.

PROGRESS: Normal and neoplastic lymphoid blood and bone marrow cells are classified using the parameters listed above. The number of proliferating and non-proliferating cells in the different subpopulations of lymphoid cells from normal individuals is compared to the number of similar cells from children with ALL or lymphoma.

285. CELL PROLIFERATION IN SOLID HUMAN TUMORS

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To obtain information on human solid tumor cell kinetics and growth fraction, tumors are perfused immediately after surgical removal in a normothermic perfusion system with an oxygenated medium containing radioactively labeled DNA precursors. Whole tumor autoradiography reveals topography and in combination with enzyme-histochemistry, cytologic specificity of proliferating and non-proliferating tumor cells. Influence of preoperative irradiation is studied with this system. The investigation started using kidney tumors and is now extended to human testicular and ovarian tumors.

286. EMBRYONAL CARCINOMA GROWTH AND DIFFERENTIATION

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Studies are progressing with the PSA-1 embryonal carcinoma cell line which differentiates to cystic embryoid bodies under cell culture conditions.

These studies involve a detailed analysis of DNA synthesis while the PSA-1 cultures are differentiating to embryoid bodies plus the selection of temperature sensitive growth mutants of PSA-1 embryonal carcinoma cells.

We are currently using an effect described by others which allows for high cloning efficiencies of PSA-1 cells in the absence of feeder cells, the addition of mercaptoethanol to the growth media. This effect is being used in our mutant work.

287. CELL CONTROL IN INTEGUMENTAL CANCER

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CANCER BIOLOGY: In vitro cell kinetics and growth potential of normal keratinocytes and fibroblasts and of the same cell types from benign and malignant hyperplastic skin disease of hyperplasia are compared. Cellular defects in these cell types, i.e., defect in DNA synthesis, response to hormones, DNA repair, mucopolysaccharide and collagen metabolism are studied. Blood levels of polypeptide hormone, epidermal growth factor, and its role in hyperplastic cutaneous disorders are determined.

CARCINOGENESIS: Carcinogenic potential of pharmacologic agents (e.g., mechlorethamine, psoralen-ultraviolet light) is monitored in laboratory animals which have received cutaneous administration of such agents and in patients receiving such agents therapeutically.

CANCER THERAPY: Therapeutic control of the lymphoma mycosis fungoides by single agents and combinations thereof is pursued. Agents include topical and systemic drug materials as well as physical agents.

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288. PATHOPHYSIOLOGY AND CHEMOTHERAPY IN PSORIASIS AND CANCER

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The epidermis of human skin is a constantly regenerating tissue which gives rise to pathologic conditions characterized by altered rates of cellular proliferation. Cell proliferation kinetics have yielded valuable information on the pathophysiology of diseases like psoriasis leading to more rational and improved forms of chemotherapy. Similar studies are being performed on other primary skin diseases as well as on malignacies that metastasize to the skin. The normal and abnormal biochemical pathways of DNA synthesis required for cell production is being studied, both in vivo and in vitro, in a spectrum of proliferative cutaneous diseases that include psoriasis, pityriasis rubra pilaris, skin cancer and cutaneous metastases of internal malignacies taking advantage of their easy accessibility.

The pharmacology of chemotherapeutic drugs is extremely important for the therapy of diseases like psoriasis, pityriasis rubra pilaris and malignancies. The mechanism of drug action in these skin diseases is being studied biochemically to find potential sites for specific drug attack. The use of methotrexate, its analogues and other chemotherapeutic drugs is continuing to be studied intensively in psoriasis with the primary objective to develop a topically effective chemotherapeutic drug.

289. LABORATORY INVESTIGATION OF TUMOR CELL BIOLOGY

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This project uses a multidisciplinary approach to study tumor cell biology so as to understand the basic nature of human malignancy and to develop methods for the diagnosis and control of human cancer. Particular emphasis is placed on lung cancer and cutaneous T-cell lymphomas. Our major efforts are in the growth of human tumors in vitro and in the nude mouse to study the differentiation, cell kinetics, immunology, experimental therapy, biochemistry, growth factor requirements, tumor markers, and ectopic hormone secretion in these model systems. The human tumor colony forming and nude mouse xenograft assays are used to study tumor biology and to test tumor sensitivity in vitro. Another major area is the use of somatic cell hybrids to study tumor cell biology and cell genetics. These include production of monoclonal antibodies by hybridomas against tumor antigens and defined proteins, comparative gene mapping, human hormone production, and genes controlling expression of the malignant phenotype. Other areas studied include tumor blood flow, tumor cell kinetics, flow cytometric analysis of human tumors, and DNA content of tumor samples.

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290. STUDIES ON THE CELL KINETICS AND MORPHOLOGICAL CHARACTERISTICS OF INDUCED INTESTINAL TUMORS

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OBJECTIVES: Most human intestinal tumors have been reproduced in animals by using DMH which is a highly efficient and organotrophic carcinogen. We have 12 years of experience in the study of factors regulating intestinal morphology. Also, most of the important morphological techniques are used in our laboratory. We intend to examine how factors regulating intestinal morphology behave during carcinogenesis.

APPROACH: Sporadic examinations of DMH-induced tumors are reported in the literature. We intend to use a systematic approach with qualitative and quantitative histological methods. This would include light and electron microscopy, radioautography, cell kinetics studies, organ culture, histochemistry. It is hoped that further details on the development of cancer will be gained by this study.

PROGRESS: The project started less than a year ago. We have followed the development of duodenal and jejunal tumors. We have described a few characteristic features of the tumors and the precancerous tissue. This included the immunohistochemical demonstration of LETS proteins. Initial radioautographic study traced DMH to the glycoproteins of cell surface and mucous cells. Some of these latter cells appeared to increase markedly in number during carcinogenesis; they also differed somewhat from conventionally described cell types. It was also inferred from the histological pictures that an antigenic or irritant factor was present in the bottom zone of tumors (near the original stem cells of the tissue).

291. HUMAN COLORECTAL TUMOR KINETICS

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In this research application the cell kinetic profile of surgically resected individual human colorectal tumors will be made at time of removal from the patient. Explants of these tumors will be made into nude mice and their kinetic properties determined in two successive transplants thereafter. The kinetics of the explants will be compared to original primary tumor kinetics to determine if alterations have occurred. This information could be extremely important since the ultimate significance of utilizing xenografts as human tumor models depends largely on the extent to which they duplicate the kinetics and response characteristics of the primary tumor. In addition the cell kinetic information obtained on the patient material will add to the data base being established in these laboratories for human colorectal tumors.

292. FBC - KINETICS OF LUNG CELLS IN RESPONSE TO INHALED EFFLUENTS

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Because cellular division and proliferation represent the primary means by which pulmonary injury is repaired, cytokinetic techniques can be used not only as sensitive indicators of lung cell damage but also to better understand patho logic proliferative processes such as pulmonary fibrosis, cellular hyperplasia, metaplasia or neoplasia. Such techniques are being used to assess the potential health effects associated with the inhalation of FBC effluent material such as trace metals and hydrocarbons. Autoratiographic methods have been developed and are providing essential baseline information on normal cell turnover times for various lung cell populations. Once these are established, animals will receive either acute or chronic inhalation exposures to selected FBC effluent products and assessed for: (1) the presence of increased cell renewal; (2) the rate at which various cell populations are being replenished; and (3) the duration of increased cell turnover following inhalation exposure. These cytokinetic data will be correlated with observed physologic and pathologic changes to assess the relationship between cell division and the development of specific pulmonary diseases.

293. STUDIES OF MONOCLONAL GAMMOPATHIES IN HUMANS

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The study is a multifaceted program consisting of four projects designed to increase our understanding of the monoclonal gammopathies. The projects and the program will increase our knowledge of the relationship between conditions associated with the monoclonal gammopathies; the mechanism of immunodeficiency in multiple myeloma; study of cell kinetics in patients with monoclonal gammopathies; and treatment of multiple myeloma bone disease with fluoride, calcium and vitamin D. These studies will provide information on this group of patients which is presently unavailable and may allow formulation of a more unified concept of these disorders.

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294. MULTIPLE MYELOMA - EVALUATION THROUGH RADIOIMMUNOASSAY

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This project concerns the development of a radioimmunoassay with which to evaluate patient response to therapy in multiple myeloma and to chart the tumor growth kinetics in this disease. This will be done by quantitating the levels of myeloma protein in serum and of Bence Jones protein in serum and urine, in a large population of patients over the course of their disease, from pretreatment to relapse. The pattern of tumor growth, remission and regrowth will be analyzed and applied to: (1) Evaluate the nature of patient response to therapy and escape from remission, (2) construct mathematical models that depict the growth kinetics of myeloma cells e.g., an exponential, Gompertzian, etc., (3) estimate tumor halving time, doubling time, and time required for proliferation of a single tumor cell to a mass sufficient to manifest the clinical syndrome, and (4) eventually, monitor new therapeutic modalities.

295. CELL KINETICS OF MULTIPLE MYELOMA (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

296. CYTOKINETICS OF HEMATOPOIETIC TUMORS

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This is part of a broader project. A summary of this subproject is not available.

IV. MECHANISMS AND CHARACTERISTICS OF TUMOR VASCULARIZATION

297. INVESTIGATION OF VASCULAR PERMEABILITY AND CEREBRAL EDEMA IN EXPERIMENTAL BRAIN TUMORS

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OBJECTIVE: A reproducible brain tumor model of short latency has recently been characterized in our laboratories. This model is a transplanted astrocytoma produced by intracerebral injection of cultured, cloned neoplastic astrocytes in the rat. It is currently being used in a study of the development of tumor vasculature. Structural and functional aspects of the blood vessels in and around the tumors are being investigated from the earliest signs of angiogenesis throughout tumor growth, with particular reference to the relationship between vasculature and: a) tumor cell proliferation and tumor malignancy, and b) the vasogenic edema associated with tumors and their abnormal vasculature.

APPROACH: The tumor vasculature and its structural abnormalities as compared with the blood vessels in normal adult brain have been characterized by light and electron microscopy. The permeability of the vascular endothelium to various specific substances is being investigated in detail, using electron-dense tracers in ultrastructural studies and radioactive materials in autoradiographic and in vivo studies on the formation and assessment of edema. Cytochemical studies on a variety of enzymes are also planned.

298. VASCULARIZATION AND BLOOD FLOW DYNAMICS IN TUMOR TISSUE

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Knowledge of tumor nutrition is of importance to the basic understanding of neoplastic progression and for permitting therapeutic advances, for instance in radiotherapy and chemotherapy. Most studies have been performed on transplanted experimental tumors and data on hemodynamics are conflicting.

DMBA-induced rat mammary neoplasia has morphological and functional similarities with human breast cancer and blood flow and vascular reactivity are under study in in vivo and in vitro systems by use of microsphere tracer technique. Improved methods for assessment of regional blood flow by use of autoradiography are being developed.

It has been found that 'resting' tumor blood flow is great, that it is nodularly distributed and that flow resistance is markedly increased upon noradrenaline infusion. A substantial fraction of blood passing the tumor is shunted past the nutritive capillaries. There seems to be no nervous control of tumor blood perfusion.

299. THE STUDY OF VASCULAR ALTERATIONS IN CEREBRAL AND CEREBELLAR METASTASES

Queiroz, A. C., Cunha Lima, C. R., Universidade Federal da Bahia, Lab. Anatomia Patologica, *c/o Hosp. Prof. Edgar Santos, Salvador, Bahia, Brazil*

OBJECTIVE: To study vascular alterations which develop in the nervous tissue peripheral to cerebral and cerebellar metastases, and to determine the significance of these alterations and possible pathogenic factors.

APPROACH: We have studied 24 cases of cerebral and cerebellar metastases. Histologic analysis of microscopic slides of the lesion is performed using hematoxylin, eosin, Masson trichrome, van Gieson and PAS stains.

PROGRESS: To date we have verified the occurrence of these alterations in 75.8% of all cases examined and in 100% of those that present with metastatic adenocarcinoma. We are currently studying the role of PAS-positive material in pathogenesis of the lesion. (Translated)

300. MALIGNANT PERFUSION - APPLIED TO DIAGNOSIS AND THERAPY

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The objective of the proposed investigation is to define the relationship between normal and malignant tumor vasculature to obtain information of potential use to cancer diagnosis and therapy in two distinct areas: (1) a study of the general characteristics of neovascularity so that manipulation of tumor blood flow can be used to increase perfusion and thus more selectively deliver tumoricidal agents (i.e. beta-emitting microspheres or chemotherapy) and diagnostic intravascular contrast agents, (2) a determination of the differences in x-ray attenuation coefficient between malignant and normal tissue using computed tomography (CT) scanning. Attempts to improve the diagnostic accuracy of CT scanning could then be systematically studied using intravascular contrast agents which could possibly be more selectively delivered to the tumor.

Host organ and tumor perfusion would be studied in high (kidney), intermediate (liver, spleen, testicle), and low (muscle) perfusion organs. Using radioactive microspheres, resting, post-vasoconstrictor and post-vasodilator (prostaglandin E1, acetylcholine), perfusion can be determined for each tumor-host organ combination. Then, after selective arterial catheterization of the tumor bearing organ and the appropriate pharmacologic intervention, chemotherpy, hyperthermia or beta-emitting microspheres would be administered and animals studied against controls for therapeutic effect.

Host tissue and malignant x-ray attenuation coefficients would be established during CT studies. A systematic study of intravenous and intra-arterial iodinated contrast agents (diatrizoate, metrizamide) with and without intravascular pharmacologic intervention would establish a standard against which to compare cancer-specific contrast agents (radiopague vesicles, polyvinylpyrrolidone, perfluorocarbon compounds) for their diagnostic usefulness.

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301. CONTROL OF TUMOR-INDUCED VASCULARIZATION

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Solid tumor growth proceeds in conncert with tumor vascularization, which requires as essential components, endothelial cells of the host capillary system and a tumor-produced mitogenic substance, the angiogenesis factor (AF). We have developed an in vitro model system, in which cultured endothelial cells from fetal bovine aorta show a stimulation in growth in response to tumor-derived substances present in the medium (Fenselau and Mello, Cancer Res. 36, 3269 (1976)). We propose to examine various aspects of the phenomenon of tumor vascularization using this in vitro model system. These features include studies on the purification and characterization of the tumor AF from the Walker 256 carcinoma, on the relationship of this agent(s) to AF from other tumors and to other known cellular growth factors, on the production and release of the tumor AF, on actual or potential antiangiogenic agents, and on the mechanisms of action by which these growth factors (both stimulators and inhibitors) affect endothelial cell growth. In sum

the thrust of these efforts is to better understand the entire process of tumor vascularization, and ultimately to be able to control this process and consequently tumor growth as well as spread.

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302. MECHANISMS INVOLVED IN TUMOR NEOVASCULARIZATION

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Proliferation of microvascular endothelium is an important event in the growth of malignant solid tumors but the mechanism(s) underlying this response are not completely understood. The present study is being undertaken to gain further insight into the mechanism(s) of tumor neovascularization by examining the potential regulatory role of tumor infiltrates, particularly monocytes and macrophages, in the initiation and/or maintenance of neovascular responses induced by tumors and to determine the significance of such an interrelationship in the control of tumor growth.

Studies will entail: 1) A kinetic study of 3H-thymidine (3H-T) incorporation by endothelial cells at various stages in the malignant progression of epithelial tumors induced by 9-10 dimethyl, 1-2 benzanthracine in the hamster cheek pouch, 2) a correlation of the onset and magnitude of endothelial 3H-T labeling with the distribution and extent of monocyte/macrophage infiltration at sites of tumor development, 3) an autoradiographic analysis of the angiogenic response to tumors in hamsters depleted of monocytes and macrophages by treatment with antimacrophage serum hydrocortisone acetate, and 4) an examination of the angiogenic potential of these and other tumor derived leukocytes following the introduction of cells and conditioned media into the avascular cornea of the guinea pig eye.

303. CAPILLARY ENDOTHELIAL CELLS AND TUMOR GROWTH

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A critical step in the development of a solid tumor is the transition of a dormant or slow growing avascular tumor into a rapidly growing metastatic vascularized tumor. This transition depends absolutely on the growth and migration of the endothelial cells that make up the capillary blood vessels. We have recently succeeded in the isolation, long-term culture and cloning of capillary endothelial cells. We have found that the controls on growth and migration of capillary endothelial cells are considerably different from those for endothelial cells derived from larger arteries and veins. We propose to employ these capillary endothelial cell cultures as a model system to study the neovascularization associated with tumor growth. We will attempt to develop an in vitro assay for angiogenesis based on the ability of an angiogenesis factor to stimulate growth and/or migration of capillary endothelial cells. These assay systems will be used in efforts to purify angiogenesis factors, to detect the presence of angiogenesis factors in tumor-bearing animals, and to screen chemotheraputic agents for their ability to inhibit tumor neovascularization.

304. NEOPLASTIC MAMMARY GLAND - STRUCTURE/ FUNCTION

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The purpose of the proposed research is to provide information about the structure and specific functions of the various cell types within normal and malignant mammary glands. (1) Mammary tissues from experimental animals (including preneoplastic lesions, hormone-dependent tumors and hormone-independent tumors) will be studied in vivo and in vitro. The fine structure and function of the different cell populations within these tissues will be elucidated, and some cellular responses to specific hormones will be characterized. (2) Human breast tissue will be maintained in organ culture and examined after exposure to specific hormones. Electron microscopy and electron microscopic autoradiography will be employed to characterize the cell types and to examine certain functional alterations that occur in the tissue (e.g., the ability of cells to divide, incorporate amino acids and bind hormones). (3) The ability of preneoplastic and neoplastic mammary tissues to elicit the ingrowth of new capillaries (a process known as mammary tumor angiogenesis) will be evaluated, and the relationship of this process to malignant transformation will be studied in hormone-dependent and hormone-independent mammary tumors. The role of hormonal regulation in tumor angiogenesis has not been studied heretofore; positive results could lead to new therapeutic methods for controlling the growth of human malignancies.

305. TUMOR ANGIOGENESIS - A CONTROL POINT IN TUMOR GROWTH

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The long-term goal of our laboratory is the exploration of three questions: (1) What is the role of angiogenesis in tumor growth? (2) How is tumor angiogenesis mediated? (3) How can tumor angiogenesis be inhibited?

The present grant is based on the second question. In our previous studies (1) capillary endothelial cells have been cloned and carried in long-term culture. (2) New methods have been developed to measure three distinct properties of capillary endothelial cells, i.e., proliferation, migration, and lumen information in vitro. (3) The sensitivity and quantitation of the chorioallantoic membrane assay for angiogenesis have been markedly improved.

We now propose to (1) study the mechanism of capillary growth by using these new in vitro methods based on capillary endothelial cell culture, and (2) use these in vitro methods as sensitive quantitation assays for tumor angiogenesis factors and for inhibitors of angiogenesis.

306. DYNAMICS OF NEOVASCULARIZATION IN SOLID TUMORS

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The two fundamental goals of this study are investigation of the morphometric law that controls the sprouting and migration of vessels in normal and neoplastic tissues and investigation of the influence of a tumor in angiogenesis and the reorganization of the vascular network during growth.

The experimental method involves implantation of tumors in Sandison type transparent ear chambers. Subsequent to tumor implantation, measurement of spatial and temporal distributions of vessel length, surface area, volume, intravessel distance, topology and branching structures are conducted. The data obtained are then generalized by developing dynamic, predictive, mathematical models, which aid in the deduction of a general morphometric law for neovascularization in normal and cancer tissue. This is the first year's funding of a three year continuing grant.

Future commitments have been made for fiscal year 1980, and fiscal year 1981.

307. ACQUISITION OF ANGIOGENIC CAPACITY BY CELL POPULATIONS UNDERGOING NEOPLASTIC TRANSFORMATION IN VITRO AND IN VIVO

Ziche, M., Gullino, P. M., Schlom, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Previous experiments have demonstrated that carcinomas as well as hyperplastic lesions at high risk of neoplastic transformation show a high frequency of angiogenic response. Present attempts aim at detecting when the angiogenic capacity appears and whether this appearance precedes neoplastic growth so that detection of increased angiogenic activity may be utilized as a marker for high risk of neoplastic transformation.

308. MASS SPECTROMETRY ANALYSIS OF COPPER IONS DURING ANGIOGENESIS IN VIVO

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Evidence has been obtained suggesting: 1) the presence of a high concentration of copper ions in purified preparations with angiogenesis activity, both from tumor extracts and normal tissues; 2) a stimulatory action of copper ions on the migration of endothelial cells in vitro and 3) angiogenic activity of PGE1. The objective of this work is to verify in vivo the role of copper ions during neovascularization induced by PGE1.

309. ISOLATION OF THE ANGIOGENESIS FACTOR(S) FROM THE INTERSTITIAL FLUID OF TUMORS

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Previous experiments on human and rodent mammary tissues have demonstrated that carcinomas and hyperplastic lesions at high risk of neoplastic transformation show a high frequency of angiogenic response. Other reports have indicated that the interstitial fluid of tumors in vivo has a strong angiogenic activity. Present attempts aim at: 1. Isolating the angiogenesis factor(s) in the tumor interstitial fluid and 2. Developing an RIA for the factor(s) as a tool for detecting high frequency to neoplastic transformation in lesions without morphological signs of malignancy.

BIBLIOGRAPHIC REFERENCES: Gullino, P. M.: Angiogenesis factor(s). In: Tissue Growth Factors. R. Baserga (ed.). New York, Springer-Verlag, 1980, in press.

310. NEOPLASTIC VASCULATURE, TUMOR GROWTH (RATS, RABBITS)

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This is part of a broader project. A summary of this subproject is not available.

V. MECHANISMS AND CHARACTERISTICS OF TUMOR INVASION AND METASTASIS

A. ROLE OF ENZYMES IN INVASION AND METASTASIS

1. CLOTTING AND FIBRINOLYTIC ENZYMES

311. ROLE OF CANCER CELL-FIBRIN INTERACTION IN METASTASIS FORMATION

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OBJECTIVE: a) To verify the possible role of the procoagulant activity of cancer cells in their metastatic behavior. b) To study the possible effectiveness of pharmacological modulation of the hemostatic system in preventing metastasis formation.

APPROACH: The problem has been studied by testing the procoagulant activity of cells derived by mechanical disaggregation of primary and metastatic tumor masses. The procoagulant activity has been studied by both a clotting (recalcification time) and an amidolytic assay; the latter utilizes a chromogenic substrate specific for activated factor X.

PROGRESS: Cells from both primary and metastatic Lewis Lung Carcinoma (3LL) have been found to possess a peculiar procoagulant activity, capable of promoting fibrin formation by directly activating coagulation factor X. Treatment of 3LL-bearing mice with warfarin at a schedule which induced marked reduction of metastasis growth was also able to inhibit the procoagulant activity of cancer cells. Such a warfarin effect appeared to be linked to vitamin K antagonism since similar inhibition of 3LL cell procoagulant activity was observed when the animals were kept on vitamin K deficient diet.

312. BIOLOGY OF SOLID TUMOR GROWTH AND IMMUNE REJECTION

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OBJECTIVES: 1. Continue and complete the characterization of the line 10 procoagulant. 2. Prepare antibodies against the procoagulant and seek to determine whether these can inhibit clotting in vitro and fibrin deposition in vivo. 3. Develop model systems in which the various tumor derived mediators may induce angiogenesis and stroma similar to that occurring about tumors in tumor-free systems in vivo.

BIBLIOGRAPHIC REFERENCES: Dickersin, G.R., Manseau, E.J., Dvorak, A.M., and Dvorak, H.F.: Fibrinous/fibrous stroma in human breast carcinoma - Possible role in tumor protection from cell mediated immunity (CMI). Lab. Invest. 1980, 42: 112. Dvorak, H.F., Orenstein, N.S., and Dvorak, A.M.: Tumor-secreted mediators and the tumor microenvironment: Relationship to immunological surveillance. In Lymphokine Reports, Vol. 2, Academic Press, New York, 1981, in press.

313. MOLECULAR AND CELLULAR STUDY OF CANCER PROCOAGULANT A

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A unique coagulation enzyme has been isolated from malignant tissue, purified to homogeneity and partially characterized. This enzyme, cancer procoagulant, has been tentatively shown to be a sulfhydryl protease that initiates clotting by directly activating factor X in the coagulation cascade. Studies of the enzymatic activity of cancer procoagulant have demonstrated that it is present in serum free medium and extracts of malignant tissue but not in the same type of samples from normal tissues.

We are continuing to study the physical, chemical and enzymatic properties of this enzyme. In addition, we are studying the role of this enzyme in the malignant process by determining its role in tumorigenicity and in the metastatic capacity of malignant cells. We have developed an antibody to cancer procoagulant and an immunoassay for the enzyme will be developed.

BIBLIOGRAPHIC REFERENCES: Stuart G. Gordon and Barbara J. Lewis. Abstract: Identification of a Unique Procoagulant Activity Associated with Malignant Transformation. Amer. Assoc. for Cancer Res. no. 716, 20-177 (1979). Stuart G. Gordon. Abstract: Comparison of Purified Cancer Procoagulant to Other Coagulation Factors That Activate Factor X. Clin. Res., Vol. 28, p. 35A, 1980.

314. STUDIES OF CANCER PROCOAGULANT A AND THE METASTATIC PROCESS

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A proteolytic enzyme, cancer procoagulant A (CPA), which is inhibited by DFP and directly activates factor X, has been purified and partially characterized. Evidence suggests the enzyme is synthesized only by malignant and transformed cells and may facilitate abnormal fibrin deposition in malignant disease. Fibrin formation is thought to participate in metastasis by facilitating the clumping of blood-borne malignant cells with other blood cells and the lodging of these clumps within capillaries of affected organs.

The objective of the proposed research will be to use clones of the B16 mouse melanoma with low (B16F1) and high (B16F10) metastatic capacity to investigate the relationship between procoagulant activity and metastatic capacity. The procoagulant activity of the intact malignant cells and serum-free medium from these cells will be quantitated and characterized. The procoagulant will be assayed using a single stage recalcified clotting time, a chromogenic substrate, and/or an immunoassay of CPA. Procoagulant activity of intact cells will be assayed with a Soniclot coagulation apparatus. Platelet aggregation ac-

tivity of purified procoagulant, serum-free medium from these malignant cells, and the intact cells will be studied using a turbidometric method. The procoagulant activity and platelet aggregation data will be correlated with the metastatic capacity.

A better understanding of the role of CPA and fibrin in the metastatic process will provide important information about the pathophysiology of metastasis.

315. TUMOR INTERACTION WITH THE FIBRINOLYTIC SYSTEM

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The purpose of this project is to investigate the relationship between tumor fibrinolytic activity and tumor metastasis formation. Pilot data has continued to demonstrate a clear-cut correlation between fibrinolytic activation and inhibition with respect to the presence or absence of tumor metastasis. During the past year we have continued to collect tumor specimans from patients undergoing palliative or curative procedures for cancer. Analysis of this data continues to support previous pilot information and further substanstiates that changes in fibrinolytic activity may form the basis for a biologic marker with respect to the presence or absence of metastatic disease. One of the critisms of our prior work was that the assay technique was quantitative but crude. During the past year we have addressed the majority of our time at efforts to improve and refine the assay technique. Using a variety of extraction techniques we have found that the fibrinolytic inhibitory activity of tumors is extractable from the tumor itself and appears to be an antiplasmin or antiplasminogen type chemical. Once this factor is extracted from the tumor cells the residual tumor ceases to show inhibitory activity. We are now in the process of doing fibrinolytic inhibitory activity separation from tumors on saphedex columns in order to further quantitate and delineate the chemical nature of the compound involved. Preliminary data suggest it is a medium molecular weight protein which is very stable. Utilzing a column separation of activators and inhibitors of fibrinolysis we are in the process of developing a vial lysis technique which can be performed with multiple samples and decreases the assay time from 8-10 hrs. to 30-240 min. Preliminary data suggests that we will be able to isolate specific activation or inhibition activity from tumors and refine and improve our assay technique so that we still have quantitative results which are more easily reproducible. Once the assay technique has been refined and tested we then will want to pursue a well-controlled animal model so that we may test the system under well defined objective controlled circumstances.

316. INFLUENCE OF ANTICOAGULATION ON METASTASIS FORMATION

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OBJECTIVE: Analysis of the process of adherence of tumor cells to the vascular endothelium: role of fibrin, evaluation of the effect of anticoagulant drugs on the distribution and survival of circulating tumor cells, analysis of mechanism of action; evaluation of possible clinical application of anticoagulative therapy in cancer treatment.

APPROACH: Four spontaneously metastasizing tumor systems in rats and mice (Lewis lung-B16-WAG rat Schwannoma-SD astrocytoma) are systematicaly screened for metastasis distribution and formation during various anticoagulant regimens (heparin, coumarins, fibrinolytic agents, etc.). The effect of anticoagulation is being studied after tumor cells are injected i.v. and transplanted s.c. Furthermore, the possibility of direct drug-tumor cell interactions will be studied.

PROGRESS: Administration of heparin or fibrinolytic agents drastically reduces the number of experimental lung tumor colonies after i.v. inoculation of tumor cells. Heparin also causes an increase in extrapulmonary, extrathoracic colonies. In contrast, spontaneous metastasis formation is not influenced. Coumarin derivatives cause a reduction in both lung tumor colonies after i.v. inoculation of cells and spontaneous metastases from s.c. transplanted tumors. Literature and experimental data indicate that the mechanism of action of coumarins is independent of coagulation factors. Restoration of the induced coagulation impairment by adding factors II, VII, IX and X inhibits the coumarin-induced reduction of the number of lung tumor colonies. Selective macrophage inhibition appeared to abolish the coumarin-induced reduction of spontaneous metastasis. Further emphasis will be placed on the analysis of the role of vitamin K and macrophages.

317. PLASMINOGEN ACTIVATOR - METASTASIS, CHEMICAL CARCINOGENESIS AND OTHER APPLICATIONS

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This application proposes: (1) To determine the requirement for plasminogen activation in embryonic development and tumor growth, metastasis, and resistance to rejection; (2) to develop fluorescent antibody methodology for detecting intracellular plasminogen activator; (3) to apply assays for plasminogen activator to the development of screening procedures for carcinogenic initiators and other agents that induce DNA damage; (4) to test whether plasminogen activator production by tumor cells under suitable conditions in culture can be used predictively as an indicator of tumor growth in vivo; (5) to compare the coordinate regulation of insulin and plasminogen activator secretion in normal and neoplastic pancreatic islet tissue; (6) to extend the clinical application and testing of Merocyanine 540, a selective supravital stain for leukemic cells.

318. ROLE OF SERINE-PROTEASE IN THE HOST-TUMOR INTERACTION

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Tumor cells in culture are able to secrete plasminogen activator and the proteolytic activity of tumor cells is believed to be connected with neoplastic behavior.

The aim of the program is to verify in vivo the presence and localization of serine enzymes in human and experimental tumors by autoradiographic technique using tritiated diisopropylfluorophosphate (3H-DFP) as a marker of serine enzymes. Proteolytic activity of tissues will be tested by the fibrin-plate technique and in the near future the isolation and characterization of in vivo labeled enzymes will be attempted.

Preliminary results obtained with human Hodgkin's and non-Hodgkin's lymphomas showed high labeling of reticular cells and epithelioid venules of lymph nodes while lymphocyte population showed low or negative labeling. Labeling was particularly heavy in lymphomas with high degree of malignancy such as immunoblastic sarcoma and centroblastic lymphoma.

The results seem to stress the importance of the host-tumor interaction and the hypothesis that reactive tissues may play a role in tumor take, growth and metastasis.

319. THE BLOOD-BORNE TUMOR EMBOLUS

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OBJECTIVES: (1) To elucidate the events which occur as tumor cells pass into and out from the vascular compartment; (2) to study the interaction of tumor cells with homologous platelets and to determine the composition of certain blood-borne tumor emboli; (3) to investigate the fate of the blood-borne embolus in certain microcirculatory units with regard to arrest, penetration of the vessel wall and metastatic development, with specific attention to methods which will test the validity of the microinjury hypothesis.

APPROACH: (1) Correlative study of scanning (SEM) and transmission electron microscopy (TEM) of human tumors, their vasculature, and the process of tumor migration into the blood vessels in the primary tumor mass. Adjacent blocks from a non-necrotic portion of the tumor mass are taken and one is further cut into small fragments and processed for TEM while the other is processed for SEM using standard methods. (2) An electron microscopic study of factors is planned to make suspensions of tumor cells from available resected tumors from the operating room and from experimental tumors in animals and to add these suspensions to platelet-rich plasma from the host animal species. TEM and SEM of thrombi containing tumor

cells will then be carried out. (3) Employing preparations of the mesentery of rats and the cheek pouch membrane of hamsters in anaesthetized animals, experiments will be designed to test the microinjury hypothesis or organ retention of circulating tumor cells. Endothelium of the capillary network will be damaged by hypertonic solutions or air embolism, the microcirculation flushed out and then suspensions of tumor cells presented to the microcirculatory unit.

BIBLIOGRAPHIC REFERENCES: Warren, B.A. Chapters 1 and 2 - Vascular Morphology of Tumors and Tumors Angiogenesis, in Tumor Blood Circulation, edited by H.I. Peterson; Cancer Letters, 4, 245-251, 1978.

320. THE ROLE OF PLATELETS IN TUMOR CELL METASTASES

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Platelets have been implicated in the establishment of tumor cell metastases: tumor cells aggregate platelets in vivo; blood-born metastases induce thrombocytopenia in the host; thrombocytopenia impairs the development of metastases; antiplatelet agents alter the metastatic pattern of tumor spread. We have extracted a sedimentable platelet aggregating material (PAM) from transformed SV3T3 fibroblast membranes with IM urea which aggregates platelet-rich plasma at concentration of 2.5 micrograms/ml. When non-transformed 3T3 cells were extracted in a similar fashion, PAM is barely detectable at 40 micrograms/ml. PAM is heat-sensitive, requires sialic acid, phospholipid and trypsin-sensitive constituents for activity, and operates via the platelet release reaction. The purpose of our proposal is to study the role of platelets in the biology of tumor cell metastasis by: 1) Characterization of the in vitro mechanisms whereby tumor cells interact with platelets; 2) Establishment of an in vivo model system to investigate the role of PAM in tumor cell spread following inoculation of animals with tumor cells; 3) Initiation of a pilot clinical project in patients with various malignancies, by screening their tumor cells to determine whether a correlation exists between their ability to aggregate platelets and the development of metastases.

A fuller understanding of the mechanisms involved in platelet-tumor cell interactions may be of help in the eventual containment and/or eradication of certain tumor metastases.

321. TUMOR CELL AND PLATELET INTERACTIONS IN THROMBOGENESIS AND METASTASIS

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Studies are proposed to further investigate the mechanism of platelet aggregation by plasma membrane vesicles spontaneously shed by mouse 15091A tumor cells and the effect of anticomplementary agents on metastases produced by this mouse tumor.

These studies involved the use of several different methods and techniques, such as: a) isolation and purification of tumor vesicles by sucrose density gradient ultracentrifugation; b) radiolabelling of normal and biochemically modified tumor vesicles; c) radioactivity monitoring of their binding to platelets in the aggregometer cuvette; d) depletion of certain complement components and IgG removal from plasma by affinity chromatography; e) biologic assay of complement; f) induction of metastases; and g) antimetastatic treatments.

2. COLLAGENOLYTIC ENZYMES

322. COLLAGENOLYTIC ENZYMES AND TUMOR INVASIVENESS

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OBJECTIVE: Penetration of connective tissues by malignant cells during metastasis or proliferation might be facilitated by the production and release of hydrolytic enzymes. We wish to test this hypothesis by studying the role of collagenolytic enzymes, especially collagenase, in relation to tumor invasiveness.

APPROACH AND PROGRESS: Using a monospecific antibody to human collagenase and immunolocalization techniques we have previously examined a variety of gastric tumors. Skin melanomas and various breast tumors are now being examined in the same way.

Immunoreactive collegenase has been detected in 4 of 16 specimens of gastric tumors and in 6 of 14 specimens of metastatic melanoma. (ref. Woolley et al., In 'Proteinases and Tumour Invasion' E.O.R.T.C. Monograph. Raven Press, in press). The cellular origin of collagenase in these specimens in still in doubt, however, and ultrastructural studies using immunoperoxidase techniques are being developed to answer this question.

The tissue distribution of the natural collagenase inhibitors alpha2-macroglobulin and beta1-anticollagenase is also being examined by immunolocalization techniques using monospecific antibodies. Preliminary studies to determine the relative importance of other collagenolytic proteinases such as cathepsin B and elastase, and also plasminogen activator, are being conducted in tumor specimens subject to tissue culture.

323. ROLE OF TYPE IV COLLAGENASE ON TUMOR METASTASES AND EFFECTS IN VIVO AND IN VITRO OF A SPECIFIC ANTISERUM

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We have already characterized and partially purified a collagenase, produced by several metastatic tumors, which specifically degrades basement membrane (type IV) collagen. We also have correlated the metastatic potential in vivo of different tumor cell lines with the production of this enzyme. We are trying to obtain an antiserum on this enzyme to better understand the mechanism of activity on the substrate and the effective role of this collagenase in the metastatic process.

BIBLIOGRAPHIC REFERENCES: Wicha, M.S., Liotta, L.A., Garbisa, S., and Kidwell, W.R. Basement membrane collagen requirements for attachment and growth of mammary epithelial cells. Exp. Cell Res. 124: 181-190, 1979. Liotta, L.A., Tryggvason, K., Garbisa, S., Foltz, K. and Shafie, S. Metastatic potential correlate with enzymatic degradation of basement membrane collagen. Nature 284: 67-68, 1980.

324. BASEMENT MEMBRANE COLLAGEN DEGRADATION AND ITS ROLE IN TUMOR INVASION AND METASTASES

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There are at least five genetically distinct collagen types whose degradation may be controlled independently. The initial step of collagen degradation is performed by collagenase. We and others have found that type IV basement membrane collagen is not degraded by human skin collagenase suggesting that a separate collagenase may degrade type IV collagen. A collagenase which preferentially degrades type IV collagen has been derived from a metastasis of the T241 fibrosarcoma. This collagenase has been purified 1000-fold and its cleavage products have been partially characterized. We are further studying the secretion rate of this enzyme by a wide variety of cell types both normal and malignant.

BIBLIOGRAPHIC REFERENCES: Murray, J.C., Liotta, L.A., Rennard, S.I., and Martin, G.R. Adhesion characteristics of metastatic and nonmetastatic tumor cells in vitro. Cancer Res. 40: 347-351, 1980. Liotta, L.A., Tryggvason, Kr., Garbisa, S., Foltz, C., and Shafie, S. Metastatic propensity correlates with tumor cell degradation of basement mebrane collagen. Nature 284: 67-68, 1980.

325. LOCAL REGULATION OF INVASIVENESS IN BLADDER TUMORS

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Malignant tumor cells are invasive by degrading such host barriers as basal lamina and connective tissues. Since collagen constitutes the primary component of these barriers, invasive-

ness must involve the degradation of collagen. We propose that this is achieved by the elaboration of specific collagenolytic activity from the invading cells. Nevertheless, certain connective tissues such as hyaline cartilage resist invasion. This selective resistance to invasion is obviously determined by tissue specific anti-invasive principles which may express themselves as a physical barrier to invasion or a system translated into the specific inhibition of the penetration by tumor cells. Indeed, cartilage contains a low molecular weight protease inhibitor (5) which is able to inhibit collagenolytic activity and which after extraction makes the cartilage penetrable to tumor cells. Similar protease inhibitors have since been found in other connective tissues including bovine urinary bladder.

In this proposal, we shall study the concept of collagenolytic activity as a proximal event in the acquisition of an invasive potential by tumor cells and define interrelationships between tumor protease (collagenase) activity, host protease (collagenase) inhibitor(s), and tumor growth characteristics in cell lines derived from chemical carcinogen (FANFT)-induced transitional cell carcinomas arising in the urinary bladder of the Fischer rat. The FANFT-model is particularly well suited for the goals of this proposal, since FANFT tumors pass through a well defined and reasonably long preinvasive stage of development before becoming invasive; thus, allowing the study of cells derived from preinvasive and invasive bladder tumors.

326. THE COLLAGENOLYTIC SYSTEM OF INVASIVE TUMORS

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Work in our laboratory has demostrated the presence of specific collagenolytic activity in invasive human and animal tumors as opposed to noninvasive benign tumors of similar origin. Neutral proteases were also found. We propose to continue the biochemical characterization of components of the collagenolytic system associated with selected invasive human and animal tumors; to correlate the level of the collagenolytic activity with tumor growth and invasion; to determine the distribution of these enzymes and their precursor molecules in the tumor parenchyma and the surrounding stroma; and to investigate the cell origin of the observed increase in enzyme activity.

It is proposed that benign tumors which have malignancy potential, may contain precursors or inactive forms of these collagenolytic enzymes which may become activated during transformation by some mechanism to be investigated. The possibility that collagenolytic enzymes of host origin may function in invasive tumor growth is being explored. The interaction between tumor and host tissue and the role of this interaction in the control of the enzyme activity is under investigation. The cell source for the tumor-associated collagenolytic activity is an important question to examine in our work. Preliminary work has shown that fibroblast-like cells derived from VX-2 carcinoma produced high levels of the collagenase activity.

Rabbit VX-2 carcinoma will continue to be used as an experimental model system for the study of these mechanisms and to test our hypothesis. It is hoped that with the full characterization of various components of the collagenolytic system and the understanding of the mechanism of control of synthesis and release of these components, tumor growth and invasion may be limited.

327. COLLAGENASE AND INVASION OF HEAD AND NECK TUMORS

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The objective of this project is to analyze and characterize a collagenolytic enzyme from a variety of head and neck tumors and to determine the significance of collagenolysis in the invasiveness and clinical behavior of these malignancies in humans and in an experimental epidermoid tumor in rats.

The current research was to compare the levels of collagenase and protease activities according to the anatomic site, histological pattern (i.e., squamous versus nonsquamous), tumor size and tumor differentiation. We have also studied the effect of prostaglandin (possibly a chemical mediator for tumor growth and spread) on the synthesis and the release of collagenase and protease. The animal model also will be used to study immunoinhibition of collagenase in vitro and in vivo to test whether immunoinhibition of collagenase will decrease tumor invasiveness and improve animal survival.

We hope that as a result of these studies, a treatment plan for head and neck tumors can be placed on a more rational basis.

BIBLIOGRAPHIC REFERENCES: Huang, C.C., Wu, C.H., and Abramson, M.: Collagenase Activity in Cultures of Rat Prostate Carcinoma. Bioch. Biophys. Acta 570:149-156, 1979.

328. ROLE OF COLLAGENASES IN TUMOR INVASION AND METASTASIS

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This proposal seeks to elucidate the role that proteolytic enzymes might play in the ability of tumors to invade normal tissue and to metastasize to other organs of the body, and has as its ultimate objective the design of specific inhibitors which will suppress malignant growth. In order to accomplish this objective, the following approaches will be taken: 1) to isolate clones of tumor cells (a methylcholanthrene-induced murine fibrosarcoma) which exhibit different degrees of metastatic potential and to see if there is any relationship between the latter property and the activities of various proteolytic enzymes, 2) to isolate and characterize those enzymes whose activities appear to correlate with metastatic potential. 3) to test the effectiveness of various synthetic inhibitors of these enzymes in curtailing the growth and invasive and metastatic properties of these tumors, 4) to conduct a detailed biochemical and cytogenetic analysis of the tumor cell population during multiple cycles of metastasis.

BIBLIOGRAPHIC REFERENCES: Wang, N., McLaughlin, M., Trend, B. and Liener, I.E. Heterogeneity of the cell population of a methylcholanthrene induced murine fibrosarcoma. J. Cell Biology 83, 108, 1979 (Abst.).

329. MAMMALIAN COLLAGENASE IN TUMOR INVASION

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Exploration of the biochemical mechanisms whereby malignant cells alter and migrate into the host's connective tissue structures is the basis of this proposal. Our studies on rabbit V2 carcinoma suggest that collagenase and hyaluronic acid may be significant components of invasive behavior of this neoplasm. We plan to extend these observations in invasive and noninvasive forms of V2 carcinoma. We will search for local and humoral factors regulating the synthesis of hyaluronate and degradative enzymes. In particular we expect to obtain more definitive evidence that these constituents do or do not contribute significantly to the invasive process.

BIBLIOGRAPHIC REFERENCES: Biswas, C. and Dayer, J-M. (1979) Stimulation of collagenase production by collagen in mammalian cell cultures. Cell 18:1035-1041. Gross, J., Highberger, J.H., Johnson-Muller, B. and Biswas C. (1980) Studies on the mode of action and regulation of tissue collagenases. In: Collagenase in normal and pathological connective tissues, eds. D.E. Woolley and J.M. Evanson; John Wiley & Sons, Ltd., Chichester, England, in press.

330. BIOCHEMICAL MECHANISMS OF CELLULAR INVASION

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The control of the production of procollagenase and plasminogen activator (PA) in cultured endothelial cells will be examined and related to the biochemical mechanisms of neovascularization.

The phorbol ester tumor promoters which induce the production of PA in a number of cell types, have been found to cause an increase in procollagenase synthesis in human umbilical

endothelial cells. To test the generality of this effect, cultures of two additional types of endothelial cells, rabbit aortic and bovine adrenal capillary cells, will be established and the effects of tumor promoters in these cells assessed. These endothelial cell cultures will then be utilized to assess a number of naturally occurring growth promoting agents for their ability to increase procollagenase and PA synthesis. Proteins such as EGF, FGF, and SGF will be tested, as well as smaller molecules, such as cyclic nucleotides, prostaglandins, and beta-adrenergics.

Since the effective level of active PA and collagenase may be controlled by mechanisms other than changes in the rate of synthesis of these molecules, the possible role of inhibitors of PA and collagenase and of fluctuations in the levels of the enzyme(s) responsible for the conversion of procollagenase to collagenase will be examined.

331. CHEMISTRY AND BIOSYNTHESIS OF CONNECTIVE TISSUE

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The purpose of this project is to study the formation, function and destruction of connective tissue components in normal and diseased states. Particular attention is directed toward collagen and proteoglycan. Current aspects of the project include (1) characterization of the matrix components in a tumor which produces basement membrane, (2) the role of collagen in diseases, and (3) interaction of tumor cells with collagens.

BIBLIOGRAPHIC REFERENCES: Foidart, J.M., Bere, E.W., Yaar, M., Rennard, S.I., Gullino, M., Martin, G.R., and Katz, S.I.: distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. Lab. Invest., 42: 336-342, 1980. Foidart, J.M., Berman, J.J., Paglia, L., Rennard, S.I., Abe, S., Perantoni, A., and Martin, G.R.: Synthesis of Fibronectin, laminin and several collagens by a liver-derived epithelial line. Lab. Invest., 42 (5): 525-532, 1980.

332. INTERACTION OF NORMAL AND MALIGNANT CELLS WITH COLLAGEN

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The phenomenon of cell movement is a major property of both embryological development and of metastatic growth, and is thought to involve recognition of the substrate by the migrating cells. Substrates of glycosaminoglycans and glycolipids are altered by migrating cells in a specific manner which indicates that the recognition system may involve a glycosyltransferase on the cell surface. This project is designed to test the possibility that surface glycosyltransferases are causally involved in cell migration, using the common extracellular component, collagen, as the substrate. The interaction of normal, malignant, and embryonic cells with collagen will be tested in three ways. First, both substrates of the enzyme (collagen and the appropriate UDP-sugar) will be provided exogenously to 3T3 cells or their transformed counterparts, 3T12 cells. Transfer of labeled sugar to collagen will be monitored, and the specificity of the reaction will be tested. Next, the UDP-sugar will be deleted to test the ability of the cells to provide their own sugar donor. Third, the specific glycosyltransferase inhibitor, UDP-dialdehyde, will be tested to see if it inhibits migration to the same extent as it inhibits glycosylation.

If the data from the 3T3 and 3T12 cells allow firm conclusions, neural crest cells will be tested in the same way since these cells undergo extensive migrations in the embryo and they also exhibit high levels of several glycosyltransferases. Migration-deficient mutants of neural crest cells will be tested to determine if any are deficient in ability to glycosylate collagen.

Little is known about the factors involved in the initiation or cessation of cell movement, except that these processes occur during abnormal, metastatic growth and during normal embryological development. This proposal is intended to collect basic information about cell migration, as well as to address a specific area of cancer cell behavior, that of metastasis.

3. OTHER PROTEOLYTIC ENZYMES AND DESTRUCTIVE SUBSTANCES IN TUMOR INVASION AND GROWTH

333. EXTRACELLULAR ROLE OF THIOL PROTEINASES IN INVASIVE TUMOR GROWTH AND METASTASIS

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OBJECTIVE: Investigation of the role of proteinases, particularly thiol proteinases, in tumor growth and metastasis.

APPROACH: The secretion of a thiol proteinase from malignant human and mouse mammary carcinomas has been observed in organ culture. Factors controlling the secretion of this enzyme are being investigated. Purification of the enzyme is being undertaken to allow biochemical characterization and to study its effect on cell surfaces and components of the intercellular matrix. The purified enzyme will also be used to produce a monospecific antiserum using either conventional or monoclonal antibody techniques. Immunolocalization studies will then be used to characterize its cellular origin.

PROGRESS: A comparative suudy of the secretion of proteinases from human mammary tumors maintained in organ culture revealed that malignant tumors secreted increased levels of a cathepsin B-like thiol proeinase when compared to fibroadenomas or normal tissue. In comparison the secretion of other proteinases - cathepsin D, collagenase and plasminogen activator - showed no significant difference between malignant and benign tumors.

A thiol proteinase similar to that secreted by malignant human tumor was also found to be secreted by spontaneous mammary tumors in C3H/j (retired breeder) mice. The enzyme from both species has enzymatic properties similar to those of cathepsin B but not unlike this lysosomal proteinase it is stable at neutral pH and has physical properties different from cathepsin B. This thiol proteinase is actively secreted from tumor tissues as its release is strictly dependent on protein synthesis. Hormone studies indicate that secretion is not affected by the presence of insulin or estradiol in the culture medium. Furthermore, there was no correlation in human tumor specimens between the capacity to secrete thiol proteinase and estrogen receptor content.

334. SYNTHETIC INHIBITORS OF TUMOR-ASSOCIATED PROTEASES

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Proteases are secreted in elevated amounts of transformed cells, a property that is correlated with tumorigenicity. Plasminogen activators are the best identified among the secreted proteases, but cathepsin B, a thiol protease, has also been observed. These proteases are thought to facilitate the ability of malignant cells to metastasize. Affinity labeling provides a means of obtaining effective and selective protease inactivators. Syntheses are proposed for several classes of affinity-labeling reagents which are expected to provide selective inhibitors of plasminogen activator without affecting closely related trypsin-like enzymes of physiologic importance and for selective inhibition of cathepsin B. Such reagents will make possible experiments to deduce the role of proteases in the pathological process and may be therapeutically promising or indicate directions for further effort.

335. A SCHEME FOR THE INVESTIGATION OF METASTATIC SPREAD OF TUMORS

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This work focuses on how the cells of malignant tumors destroy and invade surrounding tissue and on the factors affecting whether they spread to distant organs.

We have evidence that invasive tumor cells secrete substances (enzymes) which digest tissue in their path. We have also found that the surfaces of cells in secondary deposits are different from those in the parent tumor. Current experiments aim to establish whether measurement of such properties could be clinically useful in predicting the capability of a particular tumor to spread to distant organs. Detection of a marker indi-

cating high tendency to spread could be of value in individualizing treatment for cancer patients.

336. MECHANISMS OF TUMOR INVASION

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OBJECTIVE: The main purpose of this project is to gain information on the mechanisms involved in tumor invasion and tumor-induced neovascularization. In studying invasion we are especially interested in the events leading to the breakdown of extracellular matrices. Attention will be focussed on the proteolytic enzymes of host tissues and the mechanisms by which these enzymes might be induced by tumor cells. Our current work suggests that the group of enzymes most involved are metallo-proteinases.

These studies will be supported by investigations into the effectiveness of inhibitors (both natural and synthetic) of metallo-proteinases in preventing both matrix resorption and tumor-induced neovascularization.

APPROACH: The stages of the project are as follows: a) To purify and characterize metallo-proteinases and inhibitors from normal tissues, and compare them with those from invasive and non-invasive animal and human tumors. b) To investigate whether tumor cells secrete either factors that alter the normal balance of inhibitors and enzymes of invaded tissues, or proteinases capable of activating latent tissue enzymes. c) To purify and characterize the metallo-proteinase inhibitor that can be extracted from normal cartilage and determine if it is capable of preventing tumor-induced neovascularization. This extractable inhibitor is not the same as the one synthesized by cartilage in vitro.

Assays of inhibitors and enzymes will be made by methods that we have published. Testing for anti-angiogenesis will mainly be carried out using the chick chorioallantoic membrane, using purified tumor angiogenesis factor as the reference inducer.

PROGRESS: Recent studies suggest that all normal connective tissue can produce metallo-proteinases capable of degrading most of the constituents of extracellular matrices. Most tumors and tumor cells we have studied, however, do not produce such proteinases, rather they produce inhibitors. It is possible that invading cells induce host tissues to produce the necessary degradative proteinases, whilst they themselves are protected by inhibitors.

337. ROLE OF LYSOSOMAL PROTEASES IN INVASION OF THE V2 CARCINOMA OF THE RABBIT

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OBJECTIVE: To investigate the role of selected lysosomal proteases in invasion of a tumor with pronounced destructive activity on host tissues.

APPROACH: The V2 carcinoma of the rabbit shows a well reproducible mode of spread, consisting of vigorous local invasion with destruction of host tissues, and in distant spread to regional lymph nodes and lungs. The presence of relatively high amounts of proteases in bulk tumor tissue has been demonstrated in various laboratories, but a specific role of these enzymes in invasion, presuming their extracellular release, is still conjectural. In our study, particular attention is focused on three proteases, elastase, cathepsin B, and collagenase, as their combined action results in disruption and lysis of all elements of the extracellular matrix. Synthesis of these hydrolases by V2 cells is examined by biochemical assays, and attempts to demonstrate their critical intra- or extracellular localization in situ, at the tumor penetration front, are conducted by means of immunofluorescent staining methods at the light and electron microscopic level. In parallel, studies with non-immunofluorescent histochemical techniques are performed. This methodology is applied to V2 carcinoma growing in its natural host, the rabbit, as well as in the chorioallantoic membrane of the chick embryo where the whole invasion zone can be surveyed by morphological and histochemical methods.

PROGRESS: Isolation and purification of the rabbit enzymes required for the immunofluorescent staining experiments are well advanced. For cathepsin B, an independent histochemical method has been developed which is now being applied to histological localization of the enzyme under various experimental conditions of invasion.

338. MIGRATION, ADHESIVENESS AND HYDROLYTIC ENZYMES IN THE MALIGNANT PROCESS

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This is a project to examine the in vitro migrating activity, adhesiveness, and hydrolytic enzyme content of tumor cells and to correlate these characteristics with tumor invasiveness and metastasis.

A combination of in vitro and in vivo experiments will be employed. Using assays already available, tumor cell migratory activity, adhesiveness, and hydrolytic enzyme content will be determined. Alterations of tumor hydrolytic enzyme activity will be produced by selective inhibitors and by selecting variant cell subpopulations from the parent tumor cells. The effect of the various cell characteristics will be studied with regard to invasiveness of the tumor cells in vitro and the ability of the tumor cells to produce tumors and metastasize in vivo.

339. INVESTIGATIONS ON PROSTATE ADENOCARCINOMAS IN RATS

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A model system of prostate adenocarcinomas has been developed and used for studies on therapy and the phenomenon of metatasis. The patterns of spread and organotropism have been defined. Current studies involve cell receptor sites of hormones, the role(s) of VLDL and of galactosyl-transferase on the phenomenon of metastasis.

BIBLIOGRAPHIC REFERENCES: Chan, S.Y. and M. Pollard. Metastasis enhancing effect of heparin and its relationship to a lipoprotein factor. Journal of the National Cancer Institute, in press 1980. Pollard, Morris. The Pollard tumors. In: Models for Prostate Cancer (Ed.: G.P. Murphy) Alan R. Liss, Inc., New York, 1980, in press.

340. THE ROLE OF CELLULAR TRANSGLUTAMINASES IN THE GROWTH AND METASTASIS OF RAT SARCOMATA

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OBJECTIVE: To determine the activity and distribution of transglutaminases in established transplantable tumor lines in rat. The role of fibronectin as a possible substrate for this enzyme will also be investigated. It is intended to relate these factors to the proliferation rate and metastasis of malignant cells.

APPROACH: Transglutaminase activity will be assayed using C14 putrescine; localization of transglutaminase activity will be by autoradiography. Fibronectin levels and cellular distribution will be determined by fluorescein conjugated anti-rat fibronectin serum.

341. ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES OF THE INTERACTIONS BETWEEN CANCER CELLS AND NORMAL CELLS

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OBJECTIVE: Study of the nature of the interactions between cancer cells and normal cells of the host at the ultrastructural level, to clarify: a) the mechanisms of tumour invasion; role of cell mobility and release of lytic substances by cancer cells in this process; b) the role of macrophages in the host response to cancer and the mechanisms of cancer cell destruction by activated macrophages.

APPROACH: 1. Tumor invasion: a) Transplantation of Novikoff hepatoma and rhabdomyosarcoma in rat liver, skeletal muscle, kidney and lung; b) mixed cultures of cancer cells and normal cells; c) ultrastructural and cytochemical (PTA, Con A, enzymes) studies in areas of in vivo invasion or in vitro infiltration. 2. Interactions between cancer cells and macrophages: a) Specific immunization of rats by injection of cancer cells (i.p. and s.c.); b) transplantation of the tumours in normal and immunized rats; c) mixed cultures of cancer cells and peritoneal macrophages from normal and immunized rats; d) effect of

soluble factors from normal and activated macrophages on cancer cells in culture; e) colony formation by cancer cells in mixed cultures (cancer cells/normal or activated macrophages); f) ultrastructural and cytochemical studies of the interactions between cancer cells and macrophages; g) study of lytic and/or static effects of macrophages on cancer cells using thymidine and Cr release methods.

PROGRESS: a) Ultrastructural study of liver invasion by Novikoff hepatoma (Cancer Res. 32, 2765-2770, 1972). b) Etude ultrastructurale sur la pathogenie de l'invasion du muscle strie par des tumeurs transplantables (J. Ultrastruct. Res. 56, 287-303, 1976). c) In vitro interactions between normal liver cells and cancer cells: an ultrastructural study (Virchows Arch. B Cell Path. 29, 267-280, 1979).

342. OSTEOTROPISM AND HYPERCALCEMIA OF CANCER

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It is proposed to study the role of osteolytic prostaglandins in promoting cancer metastases in bone. It is the premise of this study that tumor production or tumor induced production of osteolytic prostaglandins may facilitate osteolysis, tumor implantation and tumor growth in bone. A fetal rat, explanted bone culture system will be used to study prostaglandin synthesis, bone resorption and cancer invasion. Several inflammatory and cell proliferative stimuli as well as soluble mediators derived from immune cells will be tested for their ability to induce prostaglandin synthesis and osteolysis in bone. Agents which augment or inhibit prostaglandin synthesis and release will be examined to determine their effect on tumor invasion and growth in vitro.

B. CELL SURFACE COMPONENTS AND CELL-CELL INTERACTIONS IN INVASION AND METASTASIS

343. GROWTH, METASTASES AND THE CELL SURFACE

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OBJECTIVES: Search for relevance of cell surface to growth in cultures and to invasive growth and metastasis in vivo.

APPROACH: 1. Growth in vitro: Addition of a non-toxic derivative of the carbohydrate binding lectin concanavalin A to growing SV40-transformed mouse 3T3 fibroblasts inhibited their growth at lower concentration than was required to inhibit growth of the untransformed cells. The mechanisms of action of this cell surface modulation upon growth control will be investigated by the following approaches: Determination of the point in the cell cycle when the cells are afflicted, determination of whether cell migration is also influenced, whether cell-cell adhesion is influenced, whether the binding of growth factors from the serum is prevented from taking place, etc. 2. Growth and metastasis in vivo: Variants of melanoma cells (Fiedler's F-1) will be isolated. The variants should have an altered cell surface membrane (lectin-resistant variants or mutants) and will be tested for altered metastasis after i.p. and i.m. application. After establishing the biochemical surface alteration, biological parameters which might be relevant for metastasis and altered growth behavior in vivo (adhesion, migration, protease release, deformability, escape from the peritoneum, etc.) will be studied in vitro and in vivo.

PROGRESS: 3T3 cells are arrested by succinyl Con A in G1 while SV40 cells are shifting their cell cycle pattern towards more cells being in G1. Succinyl-Con A changes the anchorage independent transformed cells reversibly to anchorage dependent cells. The mechanism is still unclear (Science 201, 824, 1978).

A wheat germ agglutinin (WGA) resistant melanoma cell line was shown to metastasize less if administered i.p. (Nature 270, 437, 1979). The surface defect could be pinpointed as a decrease in WGA surface receptors, specifically the loss of two out of 4 sialic acids in a mannose containing tetra-antennary oligosaccharide which acquired two additional fucoses.

344. COLONIZATION POTENTIAL OF MAMMARY TUMOR CELLS

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OBJECTIVE: To examine the surface properties and behavioral characteristics of mammary tumor cells and relate them to whether the cells produce distant secondary deposits on I.V. inoculation.

APPROACH: Naturally occurring murine mammary tumors are disaggregated into cell suspensions by mincing and digestion with collagenase. After washing and counting the cells an aliquot is removed for histological and electron microscopical examination and cell culture. The remainder are injected I.V. into the original tumor bearer and other recipients. The mice are autopsied 3-6 weeks later. The degree of correlation of various properties, e.g., types of cells in inoculum, mitotic rate, agglutinability, condition of glycocalyx and of cell surface antigens, with colonization behavior in vivo will be studied. Cells from metastatic deposits will be compared with those from parent primary tumors and from non-neoplastic mammary glands.

345. ROLE OF THE TUMOR CELL SURFACE IN METASTASIS FORMATION

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OBJECTIVE: Investigation of mechanisms of metastasis formation.

APPROACH: Formation of metastases by blood-borne tumor cells is studied by electron microscopy. The tumor cells are injected i.v. or added to the perfusion medium of the perfused mouse liver, which is used as the experimental model. It is attempted to influence metastasis formation with various inhibitors, pretreatments of tumor cells, etc.

PROGRESS: The invasion mechanism of lymphosarcoma cells in the liver has been described (JNCI 58(1977)399). For TA3 mammary tumor cells the mechanism was found to be the same. However, considerably fewer cells reached an extravascular position. The influence of various protease inhibitors is being tested. Cells of one TA3 subline (TA3/Ha) were often phagocytized by Kupffer cells, in contrast to another TA3 subline (TA3/St). The reason for this difference is investigated.

346. CELL SURFACE STUDIES OF METASTATIC MELANOMA

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An important area in cancer research is metastasis where tumor cells invade adjacent tissues and produce secondary tumors in distant parts of their host. Recent experimental evidence suggests that these characteristics are determined, in part, by cell surface properties. In a murine model system utilizing variant B16 melanoma cell lines it was demonstrated that cell lines with higher metastatic potential (defined by the formation of more metastases per input i.v. or s.c. tumor cells) could be derived by a regimen of passing tumors from secondary target sites into tissue culture, then reimplanting i.v. and repeating the process several times. Each successive line selected for lung, brain or ovary colonization produced significantly more metastases than parent line in target tissues. We propose to use these melanoma cell lines grown in vivo as ascites tumors or in vitro in tissue culture to determine the cellular characteristics responsible for their metastatic properties. Chemical, enzymatic and ultrastructural techniques will be utilized to study cells grown in vitro and in animals. Cell adhesive interactions will be examined by quantitative cell aggregation and attachment of radiolabeled melanoma cells, endothelial extracellular matrix and normal host blood cells. Invasion of melanoma cells will be examined in target and nontarget tissue using small tissue pieces in culture. Surface degradative enzymes (proteases and glycosidases) will be determined by quantitative assays using synthetic substrates and metabolically labelled tissue extracellular matrix. Cell surface glycoproteins and antigens will be identified and characterized using affinity chromatography on immobilized-lectins or -antibodies (produced by hybridoma techniques). Shedding of

cell surface components and plasma membrane vesicles will be studied and the components or vesicles used to build drug-encapsulated liposomes in order to direct chemotherapeutic drugs to specific organ sites where metastases are likely.

347. PLASMA MEMBRANE AND METASTASIS IN RAT MAMMARY CANCER

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The proposed research is a study of the relationship between mammary tumor cell surface properties and the tumorigenicity and metastasis of the cells in a syngeneic host. Several tumor cell variant subpopulations have been isolated from the transplantable 13762 rat mammary adenocarcinoma by cloning and selection in vitro. The metastatic variants will be characterized in vivo by analysis of tumor growth at subcutaneous injection sites, short-term cell survival and organ selectivity of tumor cell arrest following injection of (125I) iododeoxyuridine-labeled cells, and patterns of long-term metastatic growth. Characterization in vitro will emphasize aspects of cell adhesiveness, to be defined by measurements of adhesion to substratum, self-aggregation, and attachment to monolayers of rat fibroblasts and endothelial cells. In biochemical studies of cell surface macromolecular composition and organization, glycoproteins, proteoglycans, antigens, and lectin-binding proteins will be identified by radiolabeling, enzymatic dissection, immunoprecipitation, and specific ligand binding; these components will be partially characterized by gel electrophoresis, and their patterns of organization will be determined using protein cross-linking reagents. The kinetics and selectivity of tumor cell surface shedding will be studied in vitro. Several variants differing in metastatic activity will be examined, so that results of biochemical analysis and in vitro characterization can be correlated with patterns of mammary tumor cell behavior in vivo.

348. MODIFICATION OF TUMOR CELL BEHAVIOR BY LIPOSOMES

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The uptake of liposomes of defined composition by cells in vivo and in vitro provides a useful experimental method for modifying cellular composition and for introducing biologically active materials into different compartments of the cell. Fusion of liposomes with the plasma membrane and/or exchange of components between the plasma membrane and liposomes adsorbed to the cell surface will be used to introduce specific phospholipids, glycolipids and cholesterol into the plasma membrane of normal and neoplastic cells in vitro to investigate the role of these components in the expression of specific cell surface properties and their effect on cell growth behavior in vitro and in vivo. In other, similar experiments 'membrane-vesicles' isolated from the plasma membrane of tumor cells will be fused with various acceptor cells. This fusion will permit transfer of plasma membrane proteins and glycoproteins between neoplastic cells exhibiting differing invasive and metastatic behavior so that the contribution of particular membrane proteins and glycoproteins in determining specific behavioral characteristics may be identified. These experiments will provide information on how changes in plasma membrane composition and cell surface organization in neoplastic cells are related to the altered behavioral properties displayed by these cells. Experiments are also proposed to evaluate the feasibility of using liposomes containing encapsulated lymphokines as a method for activating macrophage populations in tumor-bearing animals to augment host defense against tumor cells.

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349. ROLE OF GLYCOCONJUGATES IN FORMATION OF METASTASIS

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The elucidation of the possible relationship between glycoconjugate levels and profiles (gangliosides in particular) of two rat mammary tumors (SMT-2A and MT-W9a) and the formation of metastasis is the goal of this project. SMT-2A tumor metastasizes very extensively to different organs in rats, including lungs, whereas MT-W9a does not metastasize under experimental conditions. It has been observed that SMT-2A contains about 3.5-fold more lipid-bound sialic acid than nonmetastasizing MT-W9a tumor per 100 mg of lipid extracted. Also, the ganglioside pattern of these two tumors is different. SMT-2A tumors contain relatively higher quantities of two disialogangliosides, GD2 and GD1b, trisialoganglioside GT1b and tetrasialoganglioside GQ1b. On the other hand MT-W9a contains relatively and absolute more monosialohematoside GM3. The relative amounts of monosialoganglioside GM1 and disialohematoside GD3 are also higher in MT-W9a.

Analyses of gangliosides from lungs of SMT-2A-bearing rats (54 days postimplantation), containing large numbers of macroscopic metastatic nodules, reveal that the level of lipid-bound sialic acid may be up to 4-fold higher than in normal lungs and may be even higher than in SMT-2A primary tumors. The level of lipid-bound sialic acid in larger excised lung nodules (occasionally found) and secondary tumors from axillary lymph nodes are approximately 2-fold higher than in primary SMT-2A tumors. All this very strongly suggests that primary tumor cells (or clumps of cells) with a relatively higher content of gangliosides than average may more effectively originate metastatic (secondary) tumors.

Investigation of the content and profile of gangliosides in isolated tumor plasma membranes shows that plasma membranes are enriched in total ganglioside content as compared with whole cells. The degree of enrichment in SMT-2A plasma membranes is 1.8-fold and in MT-W9a, 3.1-fold. The total ganglioside content in plasma membranes from metastasizing tumor SMT-2A is 1.8-fold higher than in plasma membranes from nonmetastasizing tumor MT-W9a.

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350. A BIOCHEMICAL BASIS FOR METASTASIS

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This project is for research to test a hypothesis to explain metastasis in biochemical terms. Invasiveness of cells within an organized tissue mass is regulated by specific interactions among the cell, and fibronectins, collagens, and proteoglycans of the protein cytoskeleton external to the cell. Recent evidence suggests that certain disialogangliosides interact with fibronectin and block cell attachment to the fibronectin-collagen complex. Transformed cells with metastatic ability generally show an absence or reduction in the amount of fibronectin at the cell surface along with a reduced capacity to bind fibronectin and a reduction or loss in higher gangliosides including the putative fibronectin receptors. The loss of gangliosides is a direct reflection of alterations in biosynthetic enzymes. Thus it is proposed that the glycosyltransferases responsible for continued formation of fibronectin receptors are repressed or rendered defective as a relatively late event in the tumorigenic cascade. As a result, the cells no longer bind fibronectin, are freed from existing mobility restraints, and are able to migrate as an important or essential first step to metastasis. Specific objective include 1) correlation of ganglioside simplification and loss of fibronectin receptors with failure to bind fibronectin and metastatic ability, 2) characterization of the sialyltransferases and other enzymes of fibronectin receptor biosynthesis, and 3) an investigation of the mech-

anisms whereby synthesis of fibronectin receptors might be regulated. Work will be primarily with animal models.

351. ROLE OF GLYCOSAMINOGLYCANS AND BINDING PROTEINS IN CONTROL OF TUMOR CELL ADHESIVE, MOTILE AND INVASIVE BEHAVIOR (ABBREV)

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OBJECTIVE: The intent of this project is to determine the molecular mechanisms of invasion, specifically the role of glycosaminoglycans and binding proteins in regulating cell adhesion and motility, and therefore, invasive properties during metastatic infiltration.

APPROACH: We are examining the glycosaminoglycan synthesis at the sites of metastasis, correlating adhesive behavior on glycosaminoglycan substrata in vitro with metastatic capabilities in vivo; investigating the role of cell surface hyaluronic acid in determining adhesive/motile behavior in vitro; characterizing glycosaminoglycan binding proteins, the connectins, their binding properties, their presence in tumor and normal tissues, and their effect on tumor cell adhesion and motility; and assessing the role of surface glycosyltransferases in tumor cell movement.

PROGRESS: To date, most information available on the involvement of glycosaminoglycans and proteins in cell movement originates from studies of embryonic tissues. Several studies suggest that neural crest is actively motile on only sulphated substrata, that they require cell surface hyaluronic acid for this behavior, and that affinity purified connectins of MW 67, 60, and 42 K bind to glycosaminoglycans, increase cell-sulphated substratum adhesion, promote cell-cell aggregation, and inhibit cell movement. Given the similarities between motile embryonic cells and metastatic tumor cells, it is predicted that both glycosaminoglycans and connectins will influence tumor cell adhesive/motile properties.

352. FACTORS DETERMINING METASTASIZING ABILITY AND METASTASIS PATTERNS TUMOR DISSEMINATION RISKS IN BIOPSY PROCEDURES (ABBREV)

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OBJECTIVE: We want to define to what extent metastasis is a clonal to selection phenomenon. We also want to define cell factors which may determine ability to metastasize and more or less specific metastasis patterns. By altering cells experimentally we try to define metastasis in relation to events in the cell cycle, cell surface characteristics, and tumor cell elasticity and mobility. We use our tumor systems to test some possible anti-metastatic drugs (e.g., interferon and platelet aggregation inhibitors).

We also use them to evaluate risks, at present only estimated by guesses, that tumors may disseminate by biopsy procedures.

APPROACH: We induced new tumors in syngeneic mice in order to increase the number of well characterized, metastasizing tumors at our disposal. We induce sarcoma and carcinoma in vitro, primarily with the objective of comparing how normal cells and tumor cells are distributed and/or destroyed in the body. For such studies we elaborate on a 125IUDR DNA-labelling procedure. We study the clearance of tumor cells injected i.v. at different doses and dose intervals, and the ensuing metastasis distribution. We perform cloning experiments in vivo and in vitro. The different clones are characterized in order to evaluate factors determining ability to metastasize.

We alter cells experimentally by enzymatic treatment and by other drugs and procedures and test their tumorigenicity and dissemination pattern. In order to evaluate if different types of biopsies, particularly fine needle-aspiration biopsies, can cause tumor spread locally or distantly, we examine tumors of different malignancy in their syngeneic hosts. The tumors are subjected to biopsy procedures and then amputated. The hosts are observed for recurrences and metastases. We also use radiolabeled cells to estimate the number of tumor cells that may disseminate by a certain biopsy procedure.

353. EXPERIMENTAL STUDIES ON NEOPLASTIC PROGRESSION AND METASTASIS

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OBJECTIVES: To select non-tumorigenic (NT), tumorigenic (T) and tumorigenic-metastizing (TM) cell variants from mouse fibroblasts in culture as a model for the sequential analysis of neoplastic progression and the study of the properties of metastasizing cells. To study the in vitro and in vivo conditions which favour the development of such cell variants and their behavior as animal tumors. To characterize some of their biological and biochemical properties, with special emphasis on membrane. To study the sensitivity of these variants to various chemical and physical agents, in vitro and in vivo.

APPROACH: Starting with a number of mouse fibroblastic cell lines (L929, C3H mouse embryo, Balbc/3T3, C3H10T1/2) a selection of cell variants with different neoplastic behavior will be carried out in vitro (selection of cell variants with different anchorage-dependence for growth, colony-formation ability in the presence of dextran sulphate and hexoses, etc.) and in vivo (determination of tumorigenesis and metastasis after different inoculation procedures, routes, and host manipulation, and the culture of such cell populations). Stress is laid on spontaneously transformed cells; chemically and virus-transformed cells will be used as comparison stages.

For the selected variants, morphology and growth characteristics in vitro and in vivo will be studied. Membrane properties will be screened by enzymatic radioiodination and SDS-gel electrophoresis, hexose-uptake assays, etc.

Drug and radiation sensitivity of the variants will be studied through in vitro and in vivo survival assays.

PROGRESS: The project started mainly with the C3H-L929 cell system; the results already obtained, together with those obtained using the BHK 21/13 cells have been recently published. From L929 cells, NT, T, and TM cell variants have been obtained. T-cells have been selected rapidly through a method involving alternant in vivo-in vitro passages. Contrarywise, lung metastasizing L929 variants were selected only after long-term in vivo transplant of L929-derived tumors. Tumorigenesis (loss of local proliferation control) and metastasis (loss of position control) have been experimentally dissociated. (Text Abridged.)

354. SELECTION AND SURFACE CHARACTERIZATION OF VIRUS-TRANSFORMED TUMOR VARIANTS WITH ALTERED METASTATIC PROPERTIES

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Recent evidence indicates that cell surface properties are important in determining the extent and location of tumor metastases. This proposal deals with the selection of virus-transformed tumor variants that (a) infiltrate into normal tissues, (b) penetrate the blood stream and form blood-borne tumor emboli, (c) arrest in specific organs, (d) infiltrate through capillary endothelial tissue, (e) develop a proper microenvironment for growth, and (f) escape host immune systems. Using several murine tumor cell lines (including SV40- and MSV-transformed fibroblasts) selection procedures will be performed in vivo and in vitro to obtain metastatic variants with altered secondary site preference, tissue infiltration, surface immunology, cell adhesion, and surface enzymology. Preliminary selection with MSV3T3 cells indicate that virus-transformed cells in vivo have a low potential for blood-borne implantation, survival and growth, but variants can be obtained by sequential selection that show high rates of pulmonary tumor colonization. A variety of chemical, biochemical, immunological and ultrastructural techniques will be used to characterize the surfaces of cells with different implantation, survival and growth properties to determine which properties are important in tumor spread to specific secondary sites.

355. THE ASSESSMENT OF NORMAL AND TUMOR CELL ADHESIVENESS BY COLLISION ANALYSIS - THE ROLE OF ADHESION IN METASTASIS

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OBJECTIVES: a) Are both normal and tumor cell preparations composed of sub-populations of cells of different adhesiveness? b) Do normal cells differ from tumor cells in the extent of their adhesiveness? c) Is there any correlation between tumor cell adhesiveness and metastasis?

APPROACH: Adhesive sub-populations of normal and transformed cells will be identified by applying the principles of collision analysis (Evans and Proctor (1978) J. Cell. Sci. 33: 17-36) to cells aggregating in a cone-plate viscometer. The high and low adhesive sub-populations will be isolated using routine separation techniques (e.g., columns) and the cells of each population will be tested for metastatic ability.

356. CELL ADHESION FACTOR - BIOLOGY AND CHEMISTRY

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A plasma membrane glycoprotein, found in the extracellular matrix of normal eukaryotes but absent following transformation, is also present in substantial quantities in normal plasma. The protein has been designated fibronectin and has been shown to mediate the adhesion of cells to a collagen substratum in vitro.

As a means of determining an in vivo function for this protein we are investigating its chemical structure and adapting the techniques of in vitro cellular adhesion to a more biologically relevant substratum, specifically basement membranes excised directly from experimental animals. These limiting membranes are known to contain substantial levels of fibronectin. It is assumed that antisera directed against fibronectin and known to inhibit cellular adhesion in vitro will provide experimental means of determining the relevance of fibronectin to cellular adhesion in vivo.

Subtilisin digestion of fibronectin under specific conditions yields reproducible patterns of digestion products. Following chromatography of the fragments on a gelatin affinity column a major polypeptide of apparent molecular weight 50,000 daltons binds to and is eluted with 4M urea from the column. This fragment is still capable of mediating adhesion of fibroblasts to collagen and therefore contains both the gelatin and cellular binding sites. Further chemical characterization of this fragment has been accomplished.

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357. CELL ADHESION PROTEINS AND MALIGNANCY

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The goal of this project is to examine the role of fibronectin in cell adhesion. The proposed work is aimed at a structural and functional characterization of fibronectin.

Studies will be carried out to optimize the methods for affinity chromatography of fibronectin. With the aid of an immunological approach, we intend to map the gene coding for human fibronectin.

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358. THE MOLECULAR BASES OF CELL ADHESION

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Alterations of the adhesive interaction between cells and components of the interstitial matrix such as collagen are doubtlessly important in the invasive and metastatic behavior of malignant cells.

Excepting the rapidly growing body of knowledge concerning fibronectin, little information exists on the molecular bases of cell-matrix interaction. Clearly, surface macromolecules other than fibronectin are involved in adhesion, as are components of the cytoskeleton, but biochemical details of these involvements are scanty. Moreover, the effects of clear cut alterations of in vitro adhesiveness on the invasive or metastatic behavior cells in situ or in vivo are unknown.

The present proposal intends to explore the molecular basis and biological consequences of altered cell-substratum adhesiveness using membrane biochemistry and somatic cell genetics as tools. We have isolated and partially characterized a series of variant clones of CHO cells which are defective in their ability to adhere to a collagen substratum; we suspect that the defect may be due to an altered surface binding of fibronectin. We intend to investigate the molecular aspects of adhesion by comparing the membrane biochemistry of the variant clones with that of wild type CHO clones. We will examine the surface glycoproteins, glycosaminoglycans and glycolipids of the membranes of both types of cells. We will also examine the role of cytoskeletal sol-gel transitions in the adhesion of both wild type and variant cells. In addition we will study the biological behavior of wild type and adhesion variant CHO cells in terms of invasiveness in the chick chorioallantoic membrane and metastasis formation in nude mice.

359. MECHANISMS OF NORMAL AND NEOPLASTIC CELL ATTACHMENT

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Despite the importance of cell-substratum interactions in determining the behavior of normal and neoplastic cells, the proteins responsible for attachment have not yet been identified directly. We have developed a method whereby lactoperoxidase is covalently coupled to polystyrene tissue culture flasks and used to radioiodinate the monolayer cell proteins that come into intimate contact with the lactoperoxidase-polystyrene surface.

In the proposed project, this system would be used to label the polypeptides in extracellular material and plasma membranes of attached cells. Lactoperoxidase will also be coupled to various natural extracellular proteins and used to label the membrane and, possibly, the intracellular proteins that interact with it. The labeled polypeptides will be analyzed on exponential gradient SDS-polyacrylamide gels and their localization will be determined by usual fractionation methods.

The data thus obtained should reveal the chain of interactions between extracellular, intramembrane and intracellular proteins in normal cells. Disturbances that accompany neoplastic transformation should also be apparent.

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360. THE SYNTHESIS AND INTERACTION OF COMPONENTS MEDIATING SPECIFIC EMBRYONIC CELL ADHESION

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The mechanisms which mediate the formation and stability of intercellular attachments are critical to understanding both normal and aberrant developmental phenomena. The relative affinity of one cell for another is presumed to change during development so that cells form a continuum of differing architectural patterns with their neighbors, culminating in morphology characteristic for a particular organ or tissue. In addition

changes in the adhesive capacity of neoplastic cells may be related to their ability to metastasize and invade surrounding tissue. Characterization of the surface structures involved in the formation of adhesions, their synthesis and turnover in the embryonic chick neural retina are the objectives of this research.

361. CELL ADHESION IN LOCALIZED AND METASTATIC MALIGNANCY

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This is part of a broader project. A summary of this subproject is not available.

362. INVESTIGATION OF TISSUE INVASION USING EMBRYONIC AND MALIGNANT CELLS IN CULTURE

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OBJECTIVE: To study cell-to-cell interactions in tissue culture as one type of tissue invades another.

APPROACH: Both normal embryonic cells and malignant cells will be used and the process of invasion by normal embryonic cells will be compared with that by malignant cells. The possibility that malignant cells may inhibit the movement of certain normal cells will also be investigated. The model system used for investigating invasion in normal tissues is that of the early chick embryo, where definitive endoblast invades the hypoblast. Preliminary studies (Sanders, Bellairs and Portch, 1978; J. Embryol. exp. Morph. 46, 187-205) have suggested that certain properties of the hypoblast play an important role in permitting this invasion. The present investigation will involve the investigation of cell adhesiveness, of cell junctions, of the cell coat, of cell surface protease activity and of LETS protein in both these normal and malignant cells during the time of invasion. This information will be correlated with the behavior of the cells towards one another when growing in tissue culture.

363. SPECIFICITY OF NORMAL AND CANCER CELL INTERACTIONS

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The objective of this research is to elucidate the sequence of events which occur when a cell or cells of one type is accepted or rejected by a cell, aggregate or tissue fragment of a different cell type. In particular, we are studying the difference in recognition processes between normal and cancer cells which can account for the differential inhibitory effect of normal and cancer cells on the aggregation of embryonic cells. We are investigating how these differentiated inhibitory effects may be mediated, especially with regard to modifiers of cell adhesion, metabolism, motility, and the cell periphery including cell surface enzymes. In addition to the aggregating system, we will use stationary culture, adhesion to artificial and cellular substrates, and collecting aggregates as 'sensor systems' for the interaction of the normal and cancer cells.

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364. PROLIFERATIVE POTENTIAL OF CULTURED EPITHELIAL CELLS

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Using epithelial cells from primary human embryonic kidney cultures (HEK) and Madin-Darby canine kidney epithelial cells, we plan to study cell interactions with substrates composed of basement membrane components or of lectins. One of the basement membrane components we are investigating is laminin, which has been isolated from a transplantable mouse tumor in the laboratory of Dr. George Martin at N.I.H. We plan to elucidate the carbohydrate composition of laminin and investigate its interaction with various lectins. We will likewise characterize the surface of the epithelial cells with respect to which lectins they bind. We will then study the mechanism of binding of epithelial cells to laminin-coated substrates by trying to block binding with lectins which interact with laminin but not to the epithelial cells and vice versa. If we can find lectins which block cell binding to substrate, this will provide information about what kinds of carbohydrate moieties, either on the cell surface or on the laminin, are involved in the cell adhesion. We will then be able to see whether the adhesion can be blocked by appropriate oligosaccharide. We are also studying the adhesion of epithelial cells to substrates covered with lectins. These interactions will serve as models for studies on the adhesion of epithelial cells to basement membrane components.

365. CELL-CELL RECOGNITION CONSTITUENTS IN HEPATOGENESIS

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Cell-cell associations and specific cell junctions are critical for tissue function. Some associations are initiated during embryogenesis by cell-cell recognition. Tumorogenesis and malignancy both require changes in normal cell-cell recognition and there is significant indirect evidence demonstrating the involvement of the cell surface in these processes. A number of steps require perturbation of normal cell-cell recognition and adhesion; two of the most apparent are the original loss of adhesion which allows dissemination of malignant cells into the extravascular or vascular space and the final recognition of an adhesion to cells of the secondary site of metastatic growth. Evidence from embryonic histogenesis indicates that normal cell-cell recognition is mediated by tissue-specific cell-surface constituents. Thus, it is reasonable to propose that defects in the function of these macromolecules may play a causal role in metastasis and perhaps other steps in tumorigenesis. This research proposes to first identify and isolate such histogenetic cell-surface constituents involved in normal chick embryo liver cell-cell recognition.

Preliminary evidence indicates that such constituents exist and that at least one aspect of their function can be rapidly assayed in vitro by the quantitation of cell aggregation. The materials will first be isolated and characterized from normal embryonic liver. Specific antibodies will be developed and utilized to investigate the presence or absence of such histogenetic surface materials on Morris hepatomas of varying degrees of malignancy and histogenetic potential. Finally, the causal role of such normal cell surface constituenis in histogenesis will be assessed directly by insertion or attachment to the surface of the hepatoma cells.

366. CELL-CELL INTERACTIONS - HUMAN MAMMARY CELLS IN CULTURE

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The effects of cocultivation of human mammary stromal and glandular cells will be studied. Epithelial, myoepithelial, and fibroblastic cells derived from normal, atypical, and carcinomatous tissues will be examined for expression of specific markers possibly correlated with malignant and metastatic potential. Specifically, cells grown alone and in mixed cultures will be evaluated for: 1) Cellular and colony morphology by light microscopy and scanning electron microscopy; 2) metabolic transfer between cells and; 3) surface proteins (LETS, large external transformation sensitive). These studies may elucidate the role of cell-to-cell interactions in the invasion of normal tissue by malignant cells and in the normal differentiation of epithelial cells for secretory activity.

367. EPITHELIAL-STROMAL INTERACTIONS OF BREAST CARCINOMA

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This proposal will apply some of the concepts of developmental biology to a study of the possible role of epithelial-stromal interactions in infiltrating ductal carcinoma of the

human breast. The general approach will be to view breast carcinoma not as an isolated abnormal entity but as a series of interactions between differing tissue types beginning with alterations of cell growth patterns and progressing through invasion and metastasis. A model in vitro system for co-culturing the breast carcinoma cells with various types of 'primitive' stromas for periods of up to 10 days will be developed. The plan is to simulate laboratory conditions which will induce the carcinoma cells to display their capacities for growth, invasiveness and differentiation in a controlled environment. These properties of the cell will be examined in detail by autoradiographic and electron microscopic methods. This type of information could enhance our knowledge of the biological properties of the cancer cells in relation to surrounding tissues. In the long run, biochemical methods could be used to define more precisely the factors which may be of importance in influencing the behavior of cancer cells under various environmental conditions.

368. CELL CONTACTS IN NORMAL AND ABNORMAL MAMMARY GLAND (MICE)

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This is part of a broader project. A summary of this subproject is not available.

C. IMMUNOLOGICAL MECHANISMS INVOLVED IN METASTASIS

369. IMMUNOBIOLOGY OF THE TUMOR-HOST RELATIONSHIP - ANALYSIS OF METASTASIS AND INTRA-TUMOR HOST CELL INFILTRATION

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OBJECTIVE: To define and characterize a new model of a rapidly metastasizing mouse tumor and analyze possible roles of host cells in affecting the metastatic process.

APPROACH AND PROGRESS: An apparent H-2 loss isoantigenic variant was obtained by sequential passaging of MDAY ascites tumor cells into an H-2 incompatible mouse strain. MDAY derives from a (AxDBA/2)F1 H-2 heterozygous (H-2KkxH-sKd, H-2Dd) genetic background. The variant was obtained by injection of MDAY cells into the DBA/2 (H-2Kd, H-2Dd) parent. The presumptive variant so obtained, called MDAY-D2, completely lacked H-2k antigens and displayed a remarkable ability to metastasize in DBA/2 or (AxDBA/2)F1 recipients: animals injected subcutaneously with 105 or 106 cells died within 20 days, and showed widespread, extensive, and distant metastases, e.g., in liver, spleen, lungs, and kidneys. The original tumor, in contrast, was poorly metastatic. Comparison of MDAY and MDAY-D2 revealed extensive differences in various cell surface alloantigens and receptors e.g., Thy-1.2, Ly-1.2, Ly-2.2, alloantigens, Fc receptors, and MDAY tumor-specific antigens. This raised doubts about the supposed variant nature of MDAY-D2. The problem was analysed further by inserting a dominant and stable artifical genetic marker into the MDAY cells, namely ouabain resistance. When MDAY OuaR cells were sequentially passed into DBA/2 hosts, the MDAY-D2 tumor which arose was found to be completely ouabain sensitive. The loss of this genetic marker was accompanied by loss of H-2k antigens and acquisition of the ability to metastasize. These results raise the possibility of some sort of genetic transfer mechanism involving a DBA/2 host cell in the origin of the high-metastatic MDAY-D2 tumor line e.g., oncogenic viral transformation or gene/chromosome transfer.

We have derived a variety of low-metastatic variant sublines of MDAY-D2 by cloning procedures, or by selection of lectin-resistant variants. These sublines were analyzed for qualitative and quantitative aspects of intra-tumor host cell infiltration, and compared to the high metastatic MDAY-D2 tumor. No significant differences were observed.

370. THE IMMUNOBIOLOGY OF TUMOR METASTASIS

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We plan to analyze: (a) whether metastatic cells possess cell-surface antigens different from those of the local tumor; (b) whether immunoselective processes participate in the control of metastatic spread, and (c) what are the mechanisms through which the local tumor exerts a suppressive effect on the growth of its metastases? Lymphocytes will be sensitized in culture on monolayers of tumor cells deriving from the local and from the metastatic populations. Cross testing of CL will indicate how general is the phenomenon of antigenic differences between the two cell populations found in the 3LL tumor. The generation in culture of antimetastatic CL will provide a starting point for experiments on adoptive immunity against metastatic progression. Tests of populations of tumors grown in immunosuppressed animals will indicate whether immunoselection plays a role in metastatic spread. We shall further study our observation that metastatic cells are more resistant to NK activity and examine whether selection of NK-resistant cells is associated with an increase in their capacity to produce metastasis. The question of whether metastatic cells differ in their capacity to produce metastasis. The question of whether metastatic cells differ in their expression of H-2 determinants is relevant to the question of the recognition of the cell surface antigens of metastatic cells, by the host syngeneic lymphocytes. Hence, studies will be made using also the T-10 tumor which was produced in an F1 hybrid to test for H-2 restrictions specific for metastatic cells, as distinct from the local cells. The suppressive effect of the local tumor on the progression of its metastases will be studied, aiming mainly at defining immunological versus nonimmunological mechanisms. The fact that acceleration of metastatic progression following excision of the local tumor takes place even in immunosuppressed animals suggests a nonimmune mechanism. The possibility that the tumor secretes factors which suppress nonspecifically cell proliferation will be investigated by testing the effects of tumor growth on hemopoiesis, liver generation and skin mitosis. Concomitantly, factors from tumor cells will be isolated and tested for their effects, in vivo, in suppressing metastasis progression in tumor-excised animals.

371. IMMUNE MECHANISMS INVOLVED IN TUMOR METASTASIS

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Our studies are intended to clarify some of the mechanisms that operate in tumor metastasis. We are investigating the role of membrane-associated components of tumors, including tumor associated antigens, tumor specific antigens and tumor specific transplantation antigens. We are also investigating the role of specific anti-tumor antibodies and of tumor specific immune complexes. We are studying the interactions between these membrane-associated and humoral components and the various immunocompetent cells involved in the immune response to tumors. Such studies have the ultimate objective of providing new information that can be applied to the prevention or cure of metastatic disease.

For these purposes we are employing a relevant animal model -chemically induced, transplantable rat mammary carcinomas, both metastatic and nonmetastatic. In initial studies we have identified and quantitated a common organ specific tumor associated antigen, apparently characteristic of our metastatic tumors. This antigen is shed by the tumors and may be functionally involved in the metastatic process.

The components of interest are being identified and isolated by specific methods which include the use of immunoadsorbents, lectin affinity adsorbents and xenogeneic antibodies specific for the various membrane-associated components. Sensitive and specific radioimmunoassays are being developed to quantitate these components. Similar sensitive assays are being developed to both detect and quantitate specific anti-tumor antibodies and the specific immune complexes.

A prime objective of these studies is to determine how, and to what extent, the various immunologically defined components are involved in the metastatic process. A second objective is to use the specific analytical procedures we are developing in the animal system as guides in developing procedures for early detection of human tumors.

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372. A PROGRAM OF STUDY OF CANCER METASTASIS

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OBJECTIVES: The objectives of this program are to determine the following: a) What are the biological and biochemical properties of tumor cells which cause them to metastasize, particularly via the lymphatics? b) What is the involvement of the immune response in metastasis and can it be used to control metastatic spread? c) Are there characteristic features of metastatic cells that can be exploited in cancer chemotherapy for their specific eradication?

APPROACH AND PROGRESS: To investigate these aspects of metastasis, an animal model system to study lymphatic metastasis has been established. Using this system, variant cell lines, with specific alterations in different cell functions, are being isolated in vitro and the effects of these alterations on metastatic potential will be determined in vivo. In addition, the sensitivity of metastatic cells to various chemotherapeutic agents will be compared to those of the primary tumor.

373. THE EFFECT OF IMMUNITY ON METASTASIS AND THE IMPORTANCE OF DIET TO TUMOR RESISTANCE (ABBREV)

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OBJECTIVE: The intention is to study: a) the influence of specific immunity on tumor cells during their passage in the blood; b) the possible connection between the relation of T- and B-cells in the regional lymph nodes, spleen and blood and the capability of transplantable tumors to metastasize spontaneously; and c) the importance of the composition of the diet of mothers during gestation and lactation to the resistance of the adult offspring against tumors.

APPROACH: For the investigation syngeneic tumor-host systems and mice with a different immune status are used. a) Blood from the right heart is aspirated at the same time as the tumor cells are injected into a tail vein. The aspirated blood containing tumor cells is injected subcutaneously or intravenously into the new hosts. b) T- and B-cells are identified by immunofluorescence. Tumors which exhibit different degrees of antigenicity and which metastasize spontaneously are used. The analyses of T- and B-cells are performed before and after resection of the tumor. c) Methoxy-substituted glycerol ethers occur in the milk of different species, including man. These substances are oral adjuvants. Methoxy-substituted glycerol ethers are incorporated into pellets and given to mothers during gestation or lactation or to the young at weaning and for 3 weeks following. The resistance of the adult offspring to tumor growth and dissemination is investigated.

374. TUMOR METASTASES AND CELL-MEDIATED IMMUNITY IN A MODEL SYSTEM IN DBA/2 MICE

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OBJECTIVE: New therapy protocols effective against metastasizing tumors require an experimental in vivo test system which allows the investigation of qualitative and quantitative problems of the development of cancer invasion and metastases. Such a model system has been established recently in our group. It consists of two related chemically induced murine tumors, the non-metastasizing parental line Eb and its metastasizing variant ESb. An unrelated chemically induced tumor (MDAY) is included for specificity controls. The following questions will be studied primarily: 1. What are the properties of tumor cells important for dissemination and metastases? 2. How does the host's immune system influence these processes? 3. Can certain components of the immune system be successfully applied to the control of metastatic spread?

PROGRESS: 1. Invasive growth into normal tissue in vitro has been found as one parameter important for tumor metastases in vivo. 2. An indication of an involvement of the immune system in tumor metastases is the different degree of macrophage infiltration of the local primary tumors of Eb and ESb. Both tumors were able to induce cytotoxic T cells. The target determinants of syngeneic cytotoxic T cells on Eb and ESb were found to be different and non-crossreactive. Target antigens of cytotoxic T cells seemed to be similar to tumor associated transplantation antigens (TATA's) as defined by protection experiments in vivo. 3. Immune T cells were found to be able to transfer protective activity into mice carrying metastasizing tumor cells.

375. THE EFFECT OF CELL-MEDIATED IMMUNITY ON THE METASTATIC SPREAD OF TUMORS

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The host cell-mediated immune response can be shown to have both a stimulatory and inhibitory effect on the metastatic spread of tumors. The congenitally athymic nude (nu/nu) mouse and rat provide useful tools with which to study this phenomenon.

We are currently studying the growth and spread of syngeneic, allogeneic and xenogeneic tumors in immune competent and incompetent (nude) mice and rats; the effects of immunosuppression and stimulation in these models are also being studied.

376. AN INVESTIGATION INTO THE POTENTIAL IMMUNOMODULATING EFFECTS OF TUMOR CELLS

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This program can be divided into two areas. The first area consists of studies to determine the role of helper T cells in the induction of delayed-type hypersensitivity (DTH). A model system has been established, using the drug cyclophosphamide, which demonstrates a requirement for helper T cells for DTH induction. Using this model system we will attempt to determine the inductive requirements of helper T cells and their relationship to other functionally similar subpopulations of T lymphocytes.

The second area proposes to examine the relationship between antigen shedding and the ability of tumor cells to metastasize.

377. IMMUNOLOGICAL AND PHARMACOLOGICAL ASPECTS OF TUMORAL AND METASTATIC CELLS

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In the first phase of this study, our attention was focused on the development of new models of the metastatic process in murine hosts. In particular, a syngeneic murine ovarian tumor has been characterized and studied showing its selective tropism for only the organs of the abdominal cavity. In addition, tumor sublines isolated from a single organ and exhibiting different spontaneous metastasizing capacities have been obtained in a transplantable murine sarcoma model. The immunogenicity, chemosensitivity and immunosensitivity of these sublines are being investigated and the obtaining of clones of cells derived from spontaneous metastasis and possessing differential disseminative properties is being pursued with other, freshly induced solid murine tumors.

378. MECHANISMS OF METASTASIS

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In recent years we have developed many spontaneously metastasizing rat mammary carcinomas with various metastatic potentials in the inbred strain of W/Fu rats. These tumors encompass the entire spectrum of metastatic situations observed in human cancer, and also have many of the biological, biochemical and immunological characteristics of human carcinomas. They invade directly into the surrounding stroma and metastasize via the lymphatics, are nonimmunogenic, and shed soluble, organ-specific glycoprotein antigen from the cell surface into their microenvironment in rough proportion to their metastasizing capacity. Such antigen is detected in the sera of rats as early as one week after implantation of the tumor, even before the graft is palpable, and its level rises with the growth of tumor. Utilizing this tumor system, we plan to study the biochemical and immunological mechanisms of metastasis, anti-

gen shedding and the effect of shed antigen on the host immune system. We also plan to study the mechanism of selective anti-tumor and anti-metastatic response of athymic nude mice against the graft of these metastasizing rat tumors, while the same T-cell deficient immunological milieu accepting and promoting the growth of syngeneic nonmetastasizing, highly immunogenic rat tumors.

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379. IMMUNOBIOLOGY OF METASTASIS

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During the next year we plan to further extend our investigations into the differences between the parent MDAY-D2 metastatic tumor and its non-metastatic variants, the LMV-3 and L61. Differences with regard to surface characteristics, metabolic variations, and the abilities of these two tumors to generate suppressor responses in challenged hosts will be assessed. Studies aimed at determining whether the generation of metastasis is a dominant genetic trait will also be undertaken, utilizing cell fusion techniques for the production of hybrids of metastatic and non-metastatic cells.

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380. ROLE OF TUMOR MACROPHAGES IN MAMMARY TUMOR METASTASIS

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The overall objective of the research project is to determine the role of tumor-associated macrophages in the metastasis of different subpopulations of mammary tumors which all arose from a single autochthonous murine mammary tumor. The specific aims of this project are to: 1) Characterize the tumor-associated macrophages obtained during the growth of different tumor subpopulations derived from a single mouse mammary tumor in terms of number, surface receptors, density and enzyme markers. 2) Describe the effect of depletion of tumor-associated macrophages on the subsequent growth and metastasis of tumor subpopulations. 3) Determine whether tumor-associated macrophages isolated from different subpopulations of mammary tumors exert an enhancing or inhibitory effect on tumor survival and ascertain its specificity.

To approach this proposed research, tumor-associated macrophages will be characterized with respect to number, as determined by the number of adherent, Fc and nonspecific esterase positive, trypsin resistant cells contained in an enzymatically disaggregated tumor cell suspension which are capable of immune phagocytosis. Density differences within the population of macrophages will also be examined using a discontinuous ficoll gradient. Macrophage-depleted tumor cell suspensions will be: 1) plated in culture and in vitro doubling time calculated and 2) reimplanted into syngeneic hosts and monitored for latency period, growth rate and metastatic potential. Finally, macrophages isolated from mammary tumors will be utilized in various in vitro assays designed to examine their effect on tumor target cells and also their ability to phagocytize.

381. IMMUNOLOGIC MECHANISMS OF TUMOR METASTASIS

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The goal of this study is to understand the basic mechanisms involving tumor metastasis. Using rat mammary adenocarcinoma, we propose to examine the immunological nature of metastasis, make a comparison of the cell mediated immunity between metastasizing and non-metastasizing tumors in rat, B-rat (thymectomized, lethal irradiation and fetal liver cell reconstituted), nude (athymic) rat and nude mice.

A variety of cytotoxic assays (Natural killer assay, mitogen induced and antibody dependent and complement mediated) will be used in rat and athymic mice to dissect above objectives. We are in the process of obtaining a breeding colony of nude rats from NCI. B-rat has been made and initial experiments of metastasis were performed.

This area of vessels is highly relevant to the understanding of metastasis in cancer patients and future improvement in the management.

D. CHEMOTAXIS AND LOCOMOTION OF TUMOR CELLS

382. CHEMOTAXIS OF TUMORS OF HUMAN AND ANIMAL ORIGIN

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This research is based on the hypothesis that the chemotactic responses of malignant tumor cells contribute to the complex series of events by which tumors metastasize. It seems likely that chemotactic phenomena are able to induce the extravascular migration of circulating tumor cells in a manner analogous to that by which they direct the migration of leukocytes towards an inflammatory focus. It may be that chemotactic phenomena determine the propensity of some tumors to metastasize selectively to sites in which the factors are produced. If it is possible to understand the mechanisms which control tumor cell chemotaxis and the relationship of this phenomenon to metastasis, it may finally be possible to apply this understanding to the rational development of means to interrupt the spread of tumors.

The relationship of tumor cell chemotaxis to the metastatic process is not completely understood. One approach being applied to this problem is to develop animal models in which chemotactic stimuli can be employed to induce the extravascular migration (and metastasis) of circulating tumor cells. Once developed, these models will be used to explore means for interfering with tumor metastasis. A second approach is to conduct a survey of the chemotactic responsiveness of a variety of normal and neoplastic cell types (including human cell) in order to study the relationships between chemotaxis and the biologic behavior of the cells. This study includes examining chemotactic responsiveness of tumor cells selected for their ability to metastasize and determining the metastatic capabilities of cells selected for chemotactic responsiveness.

It is not known why chemotactic factors for tumor cells are generated in vivo although the process appears to involve the action of proteases on substrates. It has been found recently that chemotactic factors can be detected in neoplastic and inflammatory effusions, suggesting that these factors are byproducts of inflammatory processes. It is planned to examine the ability of enzymes present in (or released from) normal tissues to generate chemotactic factors. Such information would suggest that minor tissue injury might initiate the generation of chemotactic stimuli. Studies are being undertaken to explore the molecular mechanisms of tumor chemotaxis. (Text Abridged.)

383. CHEMOTAXIS OF TUMOR CELLS

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OBJECTIVE: a) To evaluate the role played by the protein carboxyl-methylating system in cancer cell chemotaxis. b) To search for tumor cell chemoattractant in normal adult mouse tissues as a way of explaining organ selectivity in metastasis.

APPROACH: a) Chemotaxing tumor cells will be incubate with 3H methionine in the presence or absence of chemoattractant and the protein-methyl esters formed will be determined. Their stability will also be investigated. Methylation inhibitor will be used to prevent chemotaxis. b) Several normal tissues will be investigated for their capacity to attract tumor cell lines that migrate specifically to lungs and brain.

384. COMPLEMENT MEDIATED TUMOR CELL CHEMOTAXIS

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The complement system serves as an important source of chemotactic factors for tumor cells. Existing evidence indicates that a fragmentation product of C5a is the main source of chemotactic factor. The proposed study will have three overall goals. These will be to 1) examine the chemotactic factor-treated cells for alterations in physiologic function; 2) probe the structure of the C5a-related chemotactic factor and the receptors on the cells for the C5a chemotactic factor and 3) determine if treatment of animals with the chemotactic factor can alter the pattern of tumor cell localization in those animals.

385. CONTROL OF CELLULAR MOBILITY

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The proposed research will deal with the following questions: (a) Are cells of invasive tumors more mobile in aggregates than cells of non-invasive tumors? To answer this question, tumors will be produced in hamsters and invasive and non-invasive growths selected. Mobility in aggregates will be assayed as before, using the histological/autoradiographic methods previously published to determine intermixing. (b) The cellular events in the penetration of a malignant cell into a solid mass (aggregate) of cells will be examined. Specifically, penetration will be assessed quantitatively as described and the morphological features of this process detailed by scanning electron microscopy. (c) The role of extracellular matrix in control of cell movement will be studied. Specifically, collagen content and biosynthesis and collagenase activity will be examined in aggregates and correlated with cellular mobility.

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386. CONTACT BEHAVIOR OF DEVELOPING AND TRANSFORMED CELLS

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The overall objective of the proposed research is to understand how normal tissue cells and cancer cells move within organisms. The emphasis is on: A) the mechanism of cell locomotion within both embryos and adults, particularly during directional morphogenetic movements and wound closure, and B) the mechanism of locomotion of invasive cancer cells, how they make contact with each other, with normal tissue cells, and with various substrata, and how this behavior relates to the acquisition of invasiveness. These two lines of emphasis are both concerned with the properties that make for cell locomotion: adhesion to the substratum (whether it consists of other cells or of extra-cellular matrix), protrusions of the cell surface, detachment and retraction of the trailing edge, cell surface flow and plasma membrane assembly, cytoplasmic flow, microfilaments and cytoplasmic contractility, microtubules and cytoplasmic viscosity, and contact inhibitory relations between cells. The principal techniques that will be utilized are cell culture, high resolution Nomarski interference, phase contrast, and interference reflection microscopy, time-lapse cinemicrography, transmission and scanning electron microscopy, micromanipulation, drug inhibition of microtubule assembly, contractility, and energy metabolism, and labeling of contractile proteins with immunofluorescent antibodies.

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387. MECHANOPHYSIOLOGY OF LOCOMOTION IN NORMAL AND NEOPLASTIC CELLS

Haemmerli, G., Strauli, P., Strub, A. M., Felix, H., Universitat Zurich, Inst. of Pathology, Division of Cancer Research, *Birchstrasse 95, 8050 Zurich, Switzerland*

This is a continuation and expansion of our former project 'Microcinematographic and ultrastructrual analysis of locomotive behavior of rat leukemia cells.'

OBJECTVE: To analyze the interaction of adhesion and contraction as mechanisms of propulsive force generation in migrating normal and neoplastic cells.

APPROACH: The study includes three of the major functional cell types of the body, lymphocytes, fibroblasts, and epithelial cells, each type represented by normal and neoplastic populations. For each population, the mode of locomotion is described by means of 'dynamic morphology,' a method developed in our former project and consisting of the combination of microcinematography and scanning electron microscopy. The new steps, which characterize the present project, consist of recording the following properties of cells in different phases of migration: (1) Adhesion sites to the substrate, visualized by reflection microscopy, (2) spatial pattern of fibrillar organelles, visualized by high voltage-transmission electron microscopy, (3) localization of contractile proteins (actin and myosin), visualized by immunofluorescence staining at the light and electron microscope levels. The results obtained by these techniques will be integrated into a working hypothesis on the dynamic interaction of adhesive sites, contractile proteins and cytoskeleton in generating the driving force of cell locomotion. Conformity or nonconformity of this mechanism in normal and neoplastic cells will be evaluated.

PROGRESS: In view of the short duration of the project, progress achieved so far is exclusively technical.

388. MORPHOLOGY OF LEUKEMIC INFILTRATION STUDIED BY LIGHT AND ELECTRON MICROSCOPY

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OBJECTIVE: To compare by a number of light and electron microscopic techniques the interaction between the cells of two biologically different rat leukemias and several host organs, particularly the liver and the mesentery.

APPROACH: Of the two rat leukemias utilized in this study, one (L5222) has highly locomotive cells, whereas the cells of the other (BNML) are practically non-locomotive. Under various experimental conditions in vitro and vivo, this different behavior has remained unchanged over a period of several years and thus reflects inherent differences of two leukemic populations. In their host animals, both leukemias display a widespread dissemination, although with variations with regard to speed and organ distribution. It can be assumed that these patterns reflect different modes of infiltration. To check this assumption, a sequential morphological examination of key organs during leukemic dissemination is carried out, utilizing transmission and scanning electron microscopy and, where suitable, microcinematography. We expect to obtain sufficient data for an adequate description of the mode of infiltration in L5222 and BNML, respectively, and for a comparative evaluation of the role of the two chief cellular mechanisms of neoplastic invasion, locomotion and lytic action, in the two experimental models.

PROGRESS: First results, concerning the mesentery, suggest a conspicuously different infiltrative behavior of the two leukemias in this organ: While L5222 shows a disaggregative pattern of growth with locomotion of individual cells, BNML displays rather an aggregative pattern with tissue destruction, but with cellular locomotion.

389. NORMAL AND TUMOR-VIRUS TRANSFORMED CELL BEHAVIOR

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The objectives of this proposal are to determine which cellular components regulate the motility and behavior of cul-

tured normal cells and their transformed derivatives. Emphasis is placed on the function of the major components of the system of cytoplasmic fibers present in the majority of mammalian cell types including microfilaments, 10 nm filaments and microtubules. These cytoplasmic fibers are thought to be responsible for controlling many aspects of behavior including cell attachment, spreading, surface topology, shape formation, locomotion, division and contact inhibition. The long-term goal of these studies is to gain new insight into the mechanisms involved in the cellular basis of transformation to the neoplastic state which ultimately results in malignant tumor formation and metastasis. Experimental approaches involved in relating structure and function in this proposal include microscopical, physiological, immunological and biochemical techniques.

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E. OTHER STUDIES IN INVASION AND METASTASIS

390. SQUAMOUS CELL CARCINOMA - INVASION MECHANISMS

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OBJECTIVE: The mechanism of invasion of squamous cell carcinoma into normal tissue and basal laminae will be examined (1) at the leading edge of tumor in human head and neck biopsies; (2) in carcinoma in situ in biopsies and exfoliated cells and in early skin cancer of mice; and (3) in an isogeneic organ culture invasion model system consisting of cultured adult mouse epithelium and added dissociated cells from skin tumors induced in the same promotion.

APPROACH: The biopsy material will be examined by thick-section stereoscopy using the Albany 1.2 MV High Voltage Electron Microscopy (HVEM) and light microscopic (LM) immunofluorescent staining of the tonofibril network. The kinetics of invasion in the model system will be quantitated in terms of the extent, depth and speed of invasion, invasive cell proliferation and dedifferentiation of the invasive cells. The extent of formation of the tonofibril network and its intracellular distribution will be examined by LM (Aniline Blue-Orange G-stain and immunofluorescence) and stereo HVEM both for motile-invaded cells and resting-invaded cells. Keratin synthesis will be modified by retinoids, protein synthesis inhibitors and hydrocortisone. The role of lysosomal enzyme release in invasion will be studied by stereo HVEM of acid phosphatase stained cells and by addition of proteases and protease inhibitors. The relation between invasion and cell proliferation will be studied by use of metabolic and microtubule inhibitors. At higher doses, chemotherapy agents will be used to search for selective toxicity for the invasive cells.

It is hoped that this work will suggest new modes of therapy for squamous cell carcinomas.

391. MORPHOGENESIS OF THE MAMMARY GLAND WITH SPECIAL REFERENCE TO INVASIVENESS

Wolpert, L., Tickle, C. A., Harrison, C. J., University of London, The Middlesex Hospital Medical School, Dept. of Biology, *Cleveland St., London W1P 6DB, England, United Kingdom*

OBJECTIVE: To determine the cellular mechanisms involved in the epithelial invasion of the embryonic mammary gland mesenchyme during normal morphogenesis to provide insights into the invasiveness of mammary carcinomas.

APPROACH: It is proposed to determine the ultrastructural characteristics at the tips of invading epithelial tongues of developing mouse mammary glands at different stages of development. Particular attention will be given to the presence or absence of the basement membrane. The pattern of cell division in the epithelium will be examined both in vivo and in organ culture. Fibrinolytic activity of the epithelium will be assayed. The invasiveness of the epithelium will be assayed in the early chick limb bud.

392. TUMOR CELL INVASION IN CULTURE

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OBJECTIVE: To determine the manner by which a cultured human fibrosarcoma cell line is able to destroy an artificially constructed blood vessel wall and associated connective tissue proteins and to relate this process to tumor invasion. The blood vessel wall is constructed by growing endothelial cells on a pre-formed layer of smooth muscle cells and has the advantage that large numbers of replicate samples can be prepared allowing quantitative studies on the invasive process in vitro. Biochemical and microscopic studies will be done simultaneously, allowing a fuller description of the invasive process. Particular emphasis will be given to the part played by the endothelium in resisting invasion since preliminary experiments have indicated that this cell layer markedly inhibits the tumor induced destruction. Fresh explants of human tumors, as well as tumor cell lines derived from metastatic tumors, will also be examined for their abilities to destroy the vessel components. These studies, using a novel system devised by us for the construction of the vessel wall, will help to define the mechanisms used by tumor cells to infiltrate vessel walls and therefore to establish secondary tumors.

393. PROSTAGLANDINS IN BLOOD CELL-ENDOTHELIAL INTERACTION - DEVELOPMENT OF THROMBOXANE & PROSTACYCLIN RADIOIMMUNOASSAY (ABBREV)

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Prostaglandins are important modulators of vascular responses. The goal of this proposal is to elucidate prostaglandin interactions between cells circulating in the blood and the blood vessel wall, in order to define basic mechanisms governing the integrity of the blood vessel surface. To measure these interactions quantitatively, radioimmunoassays will be developed for thromboxane B2 and for 6-keto prostaglandin F1a, the stable end products of two vasoactive compounds, thromboxane A2, producted by platelets and leukocytes, and prostacyclin, produced by vascular endothelium. These radioimmunoassays, in conjunction with bioassays, will be used to study the production of prostacyclin in blood vessels, using monolayers of cultured endothelial cells, fibroblasts or smooth muscle cells as models of the vessel wall. The effects of endothelial cell contact on leukocyte reactivity, platelet adhesion and aggregation and the ability of malignant cells to produce prostaglandins will be studied. In addition, the effects of prostaglandin synthesis by platelets, leukocytes and tumor cells on prostacyclin production by endothelium and on the biochemical behavior of the endothelium will be assessed. These studies will clarify how the vascular surface is normally kept nonthrombogenic and will help to understand the mechanisms underlying the process of metastasis.

394. INVASION AND METASTASIS

Zeidman, I., University of Pennsylvania, School of Medicine, Dept. of Pathology, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

Experiments will be done to evaluate the possibility that the invasion of arteries by cells of primary cancers is prevented because arterial smooth muscle presents an unfavorable environment. Interactions between cancer cells and arterial and venous smooth muscle will be studied in vitro.

Other experiments will explore the effects of dehydration on metastasis. Studies will employ transplantable tumors in mice and rabbits.

395. SUBEPITHELIAL CONNECTIVE TISSUE IN DYSPLASIAS - IN SITU AND INVASIVE CARCINOMA OF THE UTERINE CERVIX

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OBJECTIVE: To study the behavior of subepithelial connective tissue of the uterine cervix, taking into consideration all the elements that comprise the target tissue cells, fibers, fundamental substances such as blood vessels, lymph and nerves. A comparison of normal cases with cases of inflammation, epithelial dysplasias, invasive or in situ carcinoma will be made to determine changes in connective tissue that may be due to epithelial alterations or which may develop in parallel or which permit the invasion of cancerous epithelium. Metabolic and morphologic differences in the various elements of connective tissue will be studied to identify among other modifications, those in the nerve endings which could be the primary cause of cancer, according to prior study by the authors.

METHOD: Study the composition of connective tissue and nerve endings of the uterine cervix by histologic, histochemical and immunofluorescence methods.

MATERIAL: Biopsy and surgical excisions of the ecto- and endocervix in patients with normal, dysplastic and invasive or in situ cervical carcinoma.

396. COLLAGEN AND ITS RELATIONSHIP TO TUMORS

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OBJECTIVES: To establish the role and relationship of extracellular matrix, particularly collagen, to tumor growth and metastasis.

RESEARCH PLAN: 1) The amount and type of collagen produced by oncogenic and metastatic cells are being compared to non-oncogenic cells. 2) The cell attachment of these cells to different types of collagen and connective tissue substratum are being investigated. 3) The collagen type and distribution in tumors and capsules formed by oncogenic cell lines are being studied in order to determine if the oncogenic cell or the host fibroblasts are responsible for tumor stroma production.

METHODOLOGY: Cells are grown in tissue culture and pulsed with radioactive amino acids. The collagen produced by these cultures or in tumors are extracted and purified by salt precipitation, column chromatography, and gel electrophoresis. The types of collagen are finally determined by cyanogen bromide peptide analysis. The relative collagen production is measured by purified collagenase assays or hydroxyproline production.

FINDINGS: Relative collagen production decreases in all chemically transformed oncogenic cell lines. Several cell lines, both fibroblastic and epithelial have been tested. In the case of N-acetoxy-acetylaminofluorene treated rat liver epithelial cells, the type of collagen produced is altered. The reason for the change in collagen type is being investigated. Preliminary studies show that the tumors produced by these cells contain the altered collagen molecule.

397. MECHANISM OF ANESTHESIA AND SURGERY INDUCED METASTASES

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The goal of our proposal is to define a mechanism by which commonly employed anesthetic agents and major surgical procedures can enhance the growth of a malignant tumor. Our hypothesis is that certain anesthetic agents alone and other anesthetic agents in combination with surgery impair host immunity and can result in an increase in tumor growth. Preliminary data is presented to support this hypothesis. Mice develop an increase in pulmonary metastase from Thiopental alone or after a major surgical procedure. An immunorestorative drug, Thiabendazole, given in the perioperative period can decrease the number of pulmonary metastases.

The experimental plan is based upon the delayed-type hypersensitivity response to DNCB of mice, both normal and tumor-bearing, following surgery and anesthesia. This assay will be used to determine whether the defect is in the afferent or efferent arm of the immune response. Subsequently, specific in vitro assays of afferent or efferent responses will be used to localize the immune cell population(s) or interaction(s) responsible for the immunosuppression.

Finally, an attempt will be made to specifically reconstitute anesthesia/surgery treated tumor-bearing mice with cells from mice treated with an immunorestorative agent, Thiabendazole. Efficacy of this approach will be determined by decreases in metastatic tumor growth and restoration of immune reactivity.

BIBLIOGRAPHIC REFERENCES: Simon R, Lovett EJ III, Tomaszek D and Lundy J. Electrical Stimulation of the Midbrain Mediates Metastatic Tumor Growth. Science, 209:1132-1133, 1980.

398. LEVELS OF METASTASIS INHIBITION IN PRIMARY CANCERS

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We propose to determine at which levels of the metastatic cascade malignant cells from non-metastatic primary cancers are inhibited from generating clinically overt metastases. Experiments will be made to assess the behavior of these murine tumors at each of the sequential steps of metastasis: (1) Local invasion of normal tissues, using organ culture techniques, (2) separation of cancer cells from the primary lesion using in vitro model systems, (3) dissemination to distant organs by direct sampling of tumor-draining vessels and the systemic ciculation, (4) extravasation using organ cultures, and (5) covert metastasis development as assessed by bioassay procedures. The behavior of cells from each non-metastatic tumor will be compared with that of each other, low and high metastatic murine tumors at each level. The points of divergent behavior will be identified and some of the reasons for differences in patterns of metastasis determined. These studies should provide a better understanding of the complex metastatic process as a whole and provide information relevant to which stages of metastasis offer greatest opportunities for more effective inhibition of metastases in patients with cancer.

399. METASTASIS AND INVASION BY MOUSE MAMMARY TUMORS

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This is part of a broader project. A summary of this subproject is not available.

400. CELLULAR PARAMETERS OF METASTATIC TUMOR CELLS - LONG-TERM SURVIVAL PARAMETERS FOR METASTATIC CELLS IN THE SECONDARY SITES

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No summary has been provided to the Smithsonian Science Information Exchange.

401. BIOLOGY OF METASTASIS FORMATION

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This is part of a broader project. A summary of this subproject is not available.

402. CONFERENCE ON CELL PROLIFERATION, CANCER AND CANCER THERAPY

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No summary has been provided to the Smithsonian Science Information Exchange.

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